The Journal of Laboratory and Clinical Medicine

VOL. XI ST. LOUIS, MO., MAY, 1926 No. 8

CLINICAL AND EXPERIMENTAL

EXSANGUINATION-TRANSFUSION IN THE TREATMENT OF MERCURIC CHLORIDE POISONING*

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BURMEISTER and McNally[1] have shown that the absorption of mercuric chloride from the alimentary canal occurs with great rapidity; within three minutes after the oral administration of a massive dose of the poison, sufficient absorption has occurred to produce "irrecoverable damage" to the kidney. It is an unfortunate fact that many patients suffering from poisoning by mercuric chloride are not subjected to treatment for some little time after ingestion of the mercury. It is to be assumed, therefore, that in these cases considerable absorption has taken place before the employment of measures designed to remove the mercury from the alimentary canal; furthermore, it is permissible to assume that the longer the metal remains in the blood, the greater the chance of injury to the kidneys and other vital organs. The number of chemical antidotes that have been proposed for mercuric chloride bears testimony to the universal recognition of these facts; their abandonment by the majority of clinicians, together with adverse experimental evidence that has been obtained in regard to certain of them, bears testimony to their practical uselessness.

In a recent paper, Robertson[2] has proposed a method, which he designates as "exsanguination-transfusion," for the treatment of certain forms of intoxication. While Robertson recommends this procedure especially in cases suffering from poisoning by bacterial products, nevertheless he is inclined to the belief that it may be of value in dealing with other forms of poisoning;

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Received for publication, July 25, 1925.

indeed, instances are cited where it appeared to act beneficially in poisoning by resorcinol. Rosenbloom[3] has shown that a relatively large amount of the mercury is still present in the blood as long as ten days after administration of a fatal dose. It would seem, therefore, that Robertson's method of treatment should be especially efficacious in poisoning by mercuric chloride. Even before the appearance of Robertson's paper, Burmeister[4] had made the suggestion that removal of a large part of the patient's blood and its substitution by an unpoisoned blood might be of benefit in mercury poisoning. As judged by the survival of the animals used by this author, the method is of little value; the fact that certain of his treated animals appeared to suffer less renal damage than did the controls offered him some encouragement. Burmeister's technic differed from that proposed by Robertson; moreover, considerable time elapsed between the subcutaneous injection of the mercuric chloride and the commencement of treatment: these facts appeared to justify further experimentation along similar lines.

Dogs were used exclusively in the present study. The donors, apparently healthy, adult animals, were etherized and the femoral artery exposed under aseptic precautions. A glass cannula was inserted into this vessel and the blood allowed to flow into a sterile flask, containing a sufficient amount of a 3.5 per cent solution of sodium citrate to make a final concentration of this salt of 0.35 per cent. In all but two instances, the blood was used within two hours after securing it from the donor; in these two cases, the blood was preserved in the ice box for about twenty-four hours. By a somewhat crude method, the blood of donor and recipient was matched in all cases, no instances of incompatibility being seen.

Because of its greater accuracy, the intravenous administration of the mercuric chloride was employed. Sansum[5] has shown that by this method of administration, the minimal fatal dose of mercuric chloride for dogs may be determined with great accuracy. In a large number of animals, our experience has been so in accord with his that failure of any adult dog, regardless of weight, to succumb following the intravenous injection of 4 mg. mercuric chloride per kg. body weight arouses the suspicion of error in dosage or that a portion of the fluid had escaped outside the vessel, an accident which occurred in one of the control dogs of our present series.

Six dogs were used as controls for the mercuric chloride. In three of these, under morphine-procaine anesthesia, the femoral vein was exposed, and the mercuric chloride injected from a Luer syringe, a 1 per cent solution in distilled water being employed. In two other dogs, the injections were made into the jugular vein without dissection or anesthesia; in a sixth dog, one-half the dose (2 mg. per kg.) was injected into the jugular vein as described; the following day, the remainder was injected into the exposed femoral vein. As has already been mentioned, in the case of one dog, No. 9, we had reason to believe that the needle penetrated the vein wall and that a portion of the calculated dose escaped into the perivascular tissues. As may be seen from Table I, all the animals save this one succumbed to the effects of the mercury.

TABLE I

MERCURIC CHLORIDE, 4 MG. PER KG. INTRAVENOUSLY

NO.	WEIGHT IN KG.	INJECTION SITE	RESULT	REMARKS
5	11.0	Femoral	Died in 8 days	
9	5.3	Femoral	Recovered	Injection probably extravascular
12	6.5	Femoral	Died in 2 days	
16	2.3	Jugular	Died in 15 hours	
17	4.1	Jugular	Died in 9 days	
18	8.3	Jugular-Femoral	Died in 3 days	

After receiving an intravenous injection of mercuric chloride, 4 mg. per kg., six dogs were bled and subsequently transfused with whole blood. The procedure followed in five of these animals is illustrated by the following protocol:

March 13, 1925.

Dog No. 1. Brown female, weight 6.7 kg.

2:41 P.M. Morphine sulphate, 20 mg. per kilogram subcutaneously.

3:45 P.M. Under the morphine-procaine anesthesia, the femoral vessels have been exposed; 4 mg. mercuric chloride injected into femoral vein from Luer syringe (1 per cent solution).

3:59 P.M. Three hundred c.c. of blood withdrawn from femoral artery, followed immediately by injection of 350 c.c. of blood from donor into femoral vein.

4:10 P.M. Two hundred c.c. of blood withdrawn from artery; followed immediately by injection of 250 c.c. of blood into vein.

Wound closed with sutures; dry, sterile dressing. At removal from table, dog seemed in fair condition, except for depression from morphine.

March 14, 1925.

7:45 A.M. Dog stiff in death.

In some of the other instances, smaller amounts of blood were removed; in others, larger quantities. Invariably, however, the procedure seemed to have a deleterious effect; in one instance, the dog succumbed on the table; in five others, death occurred in 10, 30, 30, and 46 hours, respectively, after treatment. In order to avoid the necessity of making an incision and introducing the possibility of infection, the sixth dog was treated as follows:

April 24, 1925.

Dog No. 24. White and brown male puppy; weight 5.3 kg.

2:42 P.M. Morphine sulphate, 20 mg. per kilogram subcutaneously.

3:04 P.M. Mercuric chloride, 4 mg. per kilogram, injected into jugular vein.

3:07-3:21 P.M. Three per cent of body weight in blood removed by means of a syringe from jugular vein.

3:28 P.M. Fifty c.c. fresh, mated blood withdrawn from jugular of donor and injected into jugular of recipient.

3:30 P.M. Sixty c.c. fresh blood injected into jugular.

3:32 P.M. Fifteen c.c. fresh blood injected. Respiration ceased; artificial respiration and cardiac massage employed to no avail.

The results on all six dogs are given in Table II.

Such results as these are not calculated to render one enthusiastic: not only did we fail to save any one of the treated animals, but the average duration of life for the three dogs that did not die on the table was less than twenty-four hours; while the average life of the controls, excepting one that died in fifteen hours, was over five days. The question immediately arises

TABLE II

RESULTS OF EXSANGUINATION-TRANSFUSION TREATMENT

NO.	WEIGHT IN KG.	$HgCl_2$ MG. × KG.	INTERVAL BETWEEN ADMINIS. $HgCl_2$ AND TREATMENT	AMT. BLOOD WITHDRAWN % BODY WT.	RESULT
1	6.7	4	14 minutes	5.6	Death in 10 hours
2	9.0	4	15 minutes	5.3	Death in 30 hours
3*	9.0	4	20 minutes	5.5	Death in 30 hours
21	6.8	4	23 minutes	9.0	Death on table
24†	5.3	4	3 minutes	3.0	Death on table

*Ether anesthesia.

†Transfused directly into jugular without dissection.

whether our technic of transfusion was not responsible for the unfavorable outcome; against this is the fact that, using a suspension of washed corpuscles, Burmeister, likewise, obtained discouraging results, as far as duration of life in his animals was concerned. In order to test this possibility, however, seven dogs were transfused as controls, they having received no mercury. Except for this omission, the procedure was the same as that followed in the case of the poisoned dogs receiving the exsanguination-transfusion treatment. Of these seven controls, the operative wound became badly infected in two cases, leading to the death of the animals on the fourth and the seventh days respectively; a third dog seemed to be doing well following transfusion, but suffered a fatal hemorrhage on the fifth day following the operation. The remaining four dogs seemed in no way injured by the transfusion and were in excellent condition at the end of 8, 8, 11, and 14 days, respectively, when they were used for other purposes. In no single instance did we observe the sudden death which was seen in the case of two of the mercury dogs; indeed, the deaths that occurred in these controls were only indirectly connected with the transfusion.

It may be suggested that the mercury so weakens the resistance of the animals that have received a fatal dose of it that they are unable to resist injuries which a normal dog withstands. This is possible, but it seems unlikely that weakening to any great extent would occur even in twenty-three minutes, the longest interval that elapsed between the administration of the mercuric chloride and the institution of treatment in our dogs. Whatever the explanation may be, it seems undoubtedly proved that the exsanguination-transfusion method of treating mercuric chloride poisoning is distinctly injurious.

For a number of years, the opinion has been held by certain observers that there is a relationship between the hydrogen-ion concentration of the blood and the functional capacity or even structural integrity of the kidney.[6] The possible importance of this relationship has been recently stressed by MacNider,[7] in connection with poisoning by uranium and by mercury; in their enthusiasm, other authors have drawn conclusions from MacNider's work which are scarcely justified. Thus Weiss[8] apparently attaches great importance to the administration of alkali intravenously to patients suffering from mercuric chloride poisoning. Rosenbloom[9] also recommends the use of alkaline treatment in these cases. It has been pointed out[10] that the

administration of considerable amounts of sodium bicarbonate to dogs that had received a fatal dose of mercuric chloride intravenously did not appear to exert any favorable influence, as judged by the duration of the animal's life or by the degree of urea retention in the blood; it was felt, however, that the use of alkaline solution intravenously after the removal of a considerable amount of the animal's blood might prove more efficacious.

Three dogs were given 20 mg. morphine sulphate per kilogram subcutaneously; the femoral vein was exposed under procaine anesthesia, and 4 mg. mercuric chloride per kilogram body weight injected into this vessel from a Luer syringe. In from seven to fifteen minutes after injecting the mercury, the animals were bled from 3 to 3.9 per cent of their body weight. Immediately afterward, they received intravenously either 1 per cent or 5 per cent bicarbonate of soda solution. A fourth dog was given the mercury and, subsequently, the bicarbonate, in the form of a 5 per cent solution, without the preliminary bleeding. As may be seen from Table III, in no instance were we able to save the life of a dog by this procedure; death occurring in from ten hours to three days after the injection of the mercury.

TABLE III

RESULTS OF BLEEDING-BICARBONATE TREATMENT IN MERCURIC CHLORIDE POISONING

NO.	WT. KG.	$HgCl_2$ MG. × KG.	INTERVAL BETWEEN ADMINIS. $HgCl_2$ AND TREATMENT	BLOOD WITHDRAWN % OF BODY WEIGHT	PERCENTAGE SODIUM BICARBONATE	DEATH AFTER
7	11.0	4	7 minutes	3	1*	48 hrs.
11	11.9	4	15 minutes	3	1*	10 hrs.
23	19.6	4	13 minutes	3.9	5*	3 days
22	7.4	4	9 minutes	0	5†	3 days

*These dogs received slightly more of the bicarbonate solution than the volume of blood removed.

†This dog was given 20 c.c. of 5 per cent bicarbonate solution per kilogram.

CONCLUSIONS

1. The dose of 4 mg. of mercuric chloride intravenously is fatal to the great majority of dogs, unless there is reason to suspect faulty technic.

2. After the injection of 4 mg. mercuric chloride per kilogram intravenously into dogs, the removal of large amounts of blood and the subsequent injection of a compatible blood from another animal or of a solution of bicarbonate of soda, 1 to 5 per cent, appears to have not the least value, as judged by the early death of the animals treated in this manner.

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STUDIES ON NUTRITION IN TUBERCULOSIS*

II. Experimental Tuberculous Infection in the Albino Rat and the Influence of Vitamin Deficient Diets Thereon

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THAT nutrition plays an important rôle in the prevention and treatment of tuberculosis has long been recognized by clinical observers. Nearly all writers on the subject of tuberculosis emphasize the importance of diet in the treatment of this disease. Statistics on malnutrition and tuberculosis prevalence seem clearly to point to an intimate relationship of cause and effect rather than of mere coincidence. Though it is generally conceded that malnutrition influences tuberculosis unfavorably and may be the cause of increased tuberculosis prevalence, the converse cannot be accepted without proof, which is still lacking. Even the generally accepted premise that malnutrition affects tuberculosis unfavorably lacks precision of definition, for it is not known whether it is insufficiency of food in general or inadequacy of one or more of known specific dietary constituents that is the important causative factor in lowering bodily resistance.

Stimulated by the researches on the fat-soluble food accessory in its relation to calcium metabolism, some studies were undertaken several years ago on the influence of this vitamin on experimental tuberculosis of the guinea pig.[1]† Because of the technical difficulties involved in carrying out nutrition experiments upon the guinea pig, the results were not conclusive, but were sufficiently suggestive to encourage further work upon a more suitable laboratory animal. After some consideration the albino rat was selected for this purpose.

The choice of this animal was made for the following reasons: The nutritional requirements of the white rat have been thoroughly studied in recent years. This animal is omnivorous in its dietary habits, and the vari-

*A preliminary communication of this work was presented before the Pathological Society of the Federation of American Societies for Experimental Biology, St. Louis, December, 1923; and before the Pathological Section of the National Tuberculosis Association, Atlanta, May 9, 1924.

From Division of Pharmacology, Hygienic Laboratory, Washington, D. C.
Approved for publication by the Surgeon General, U. S. Public Health Service.
Received for publication, August 3, 1925.

†The reader is referred to these papers for a brief survey of the literature on the subject under discussion.

ous dietary factors can be controlled more or less completely. Because of the small size of the animal and the relatively small amount of food it consumes, large numbers can be cared for in a limited space, and the feeding of synthetic diets consisting of purified foodstuffs, which would otherwise be well-nigh impossible, can be accomplished without much difficulty. The rat is very susceptible to vitamin deficiencies, especially fat-soluble vitamin *A* and water soluble vitamin *B*. Its susceptibility to tuberculosis, on the other hand, would not seem to be very great, judging from some of the reports in the literature. It seemed quite reasonable, therefore, to attempt to break down the natural resistance of this animal to tubercle infection by depriving it of some of the dietary accessories which are known to be so vital in its nutrition and metabolism.

EXPERIMENTAL TUBERCULOSIS IN THE WHITE RAT

The information gathered from the literature on experimental tubercle infection in the albino rat is not altogether satisfactory. Vagedes,[2] working apparently with human strains of tubercle bacilli, stated that intravenous injection in the rat produced a few pulmonary tubercles in two months. Aoki,[3] in 1913, tested the virulence of several human and bovine strains by intraperitoneal injection in the rat, and concluded that the human strain was more virulent than the bovine for this species. It is stated that the lungs were the seat of involvement, exclusively.

Galli-Valerio[4] describing the effects of the tubercle bacillus in the white rat states that either the human or bovine strain is slow in developing in this animal, and in either case almost always produces pulmonary lesions. Enlargement of the spleen was also noted, though bacilli, it is stated, could not be found in either the spleen or liver.

From the work of Lewis and Margot[5] on the spleen in relation to tuberculosis resistance it would appear that the bovine bacillus injected intraperitoneally in the rat produces a splenic tumor, often hemorrhagic, and that the infection runs a rather rapid septicemic course.

Boquet and Negee[6] have stated that white rats are but little susceptible to tuberculosis. It is stated that 1 to 2 mg. of human, bovine, or avian bacilli injected intraperitoneally in the albino rat provoke lesions in the lungs, with multiplication of bacilli in other organs, which, however, may not show any lesions. The animals rarely succumb to the infection.

The British Royal Commission on Tuberculosis investigated this question, and their extensive reports may be briefly summarized as follows: Rats appear to be quite refractory to subcutaneous inoculation of bovine bacilli, but are more susceptible to intraperitoneal injections, the smallest dose being about 5 mg. Following such inoculation the rat dies as a result of the multiplication of bacilli in its tissues, but there is no development of the characteristic tubercle as seen in other animals. The tubercle bacillus is intracellular, but produces little tissue destruction. Similar results were also obtained with the human strain.[7, 8, 9]

In a recent publication Gloyne and Page[10] have contributed much infor-

mation on the reaction of the albino rat to *B. tuberculosis*. A virulent human strain was used in their experiments, the inoculation being made subcutaneously or intraperitoneally. The tissue changes following such inoculation are described as consisting of collections of phagocytes containing the bacilli, found for the most part in the spleen, although some were also found in the

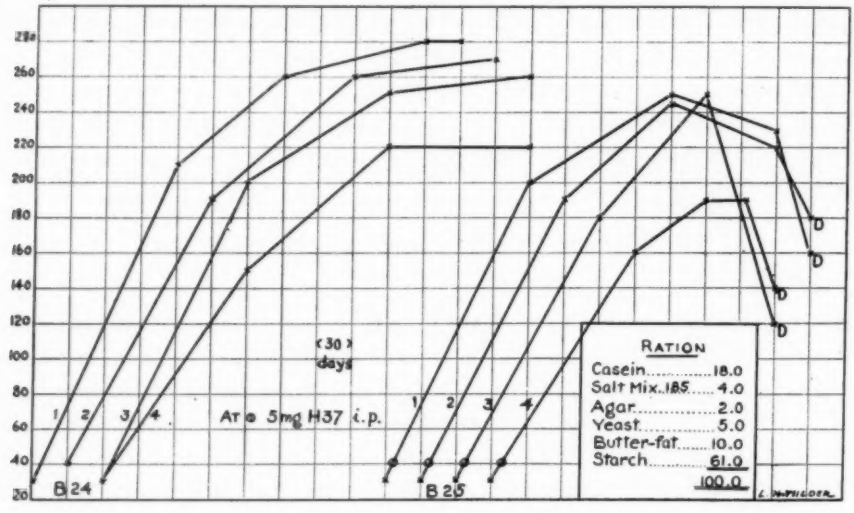

Chart. I.—Shows the course of tubercle infection in the white rat, maintained on a diet adequate in all respects. Group *B* 24 represents the weight curves of four control male rats, and group 25, of four males infected with B. tuberculosis (H 37). It will be seen that the infected animals on the adequate ration made a nearly normal growth, during the maximum growth period of about 8 months, then declined rather abruptly, death occurring about a year after infection.

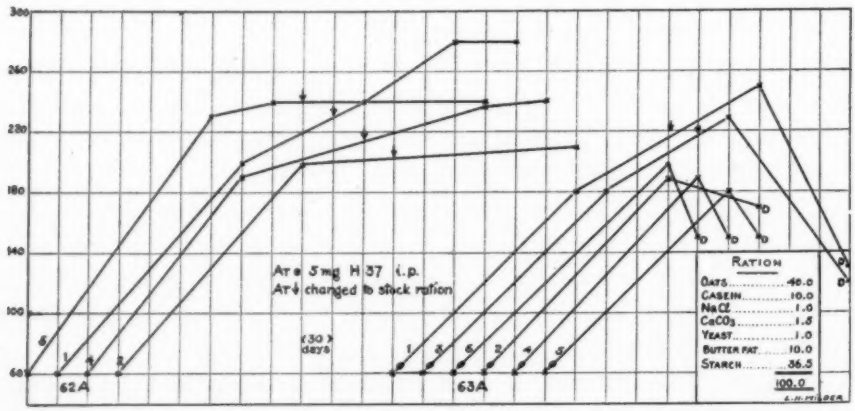

Chart II.—Course of tuberculous infection in the white rat on a diet of oats supplemented with casein, butter, yeast, and salts. Several animals in these groups developed respiratory symptoms which probably hastened death of some of the animals of the infected group. The synthetic diet was replaced by the stock ration at the point indicated in the chart. The results shown in this chart agree in the main with those of Chart I.

lungs, lymph glands, bone marrow and liver. Frequently the conglomerate masses of phagocytes presented a striking resemblance to true giant cells, though lacking the characteristic peripheral grouping of nuclei. Caseation was not noted. The masses of phagocytes are regarded by the authors as the result of endothelial proliferation, originating probably from the lymph

vessels. Curiously enough, tubercle bacilli were only occasionally found in the endothelial cells, but were most commonly seen in the "giant cells." Phagocytosis appeared to be the most marked feature in the process, and attempts were therefore made to increase the susceptibility of the rat to tubercle infection by means of various agents tending to inhibit phagocytosis. The results were negative. Experiments on sensitization of inoculated rats to tuberculin also failed to yield results. The work of these authors is referred to at some length, because the experiments described herein were carried out, at about the same time, and some of the results obtained by us are somewhat at variance with those of Gloyne and Page.

A virulent human strain, H 37 grown on glycerin agar or glycerin broth, was used in the present experiments. Emulsions of 5 mg. of moist weight per cubic centimeter in sterile physiologic salt solution were prepared, and doses of one cubic centimeter of the emulsion were injected intraperitoneally. The lesions produced by this treatment in young animals maintained on an adequate diet become clearly manifest in from thirty to sixty days, and progress till the death of the animal. The length of life in such infected animals usually runs from ten to fifteen months (see Charts I and II). Animals infected and maintained on an adequate diet, such as

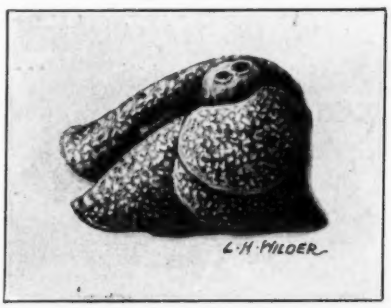

Fig. 1.—Appearance of lungs of rat infected with B. tuberculosis, human strain (H 37). Animal was killed about 6 months after infection. The lungs are voluminous, do not collapse, and are studded with grayish white nodules.

are shown in Charts I and II, show no evidence of ill health for many months, run a nearly normal weight curve throughout their growth period of about six to eight months, but soon thereafter begin to decline rather abruptly. During the period of decline the animals show some embarrassment of respiration, loss of appetite, apathy, emaciation, and a moderate degree of cyanosis. Some animals may live with such symptoms for several months and show an extreme degree of cachexia, though as a rule, when once developed these symptoms become aggravated rather rapidly, the animals appear in ill health, and die within a month or two.

The autopsy findings are emaciation and loss of subcutaneous and other body fats. The spleen is generally much enlarged and congested. The lungs are voluminous, do not collapse, and are mottled with grayish yellowish conglomerate "tubercles." (Fig. 1.) These nodules are so numerous in the advanced cases that there is hardly any normal lung tissue left. The lung

lesions are of constant occurrence and, together with the enlarged spleen, constitute the most striking feature macroscopically.

The tubercle bacillus was isolated on several occasions from the pulmonary lesions, and guinea pigs inoculated with either the culture or with the infected lung material proved the pathogenicity of the recovered microorganism.

The histologic findings are briefly as follows: As early as six days after inoculation there is some cellular proliferation in the omentum with the formation of large multinucleated eosinophilic cells presenting an appearance

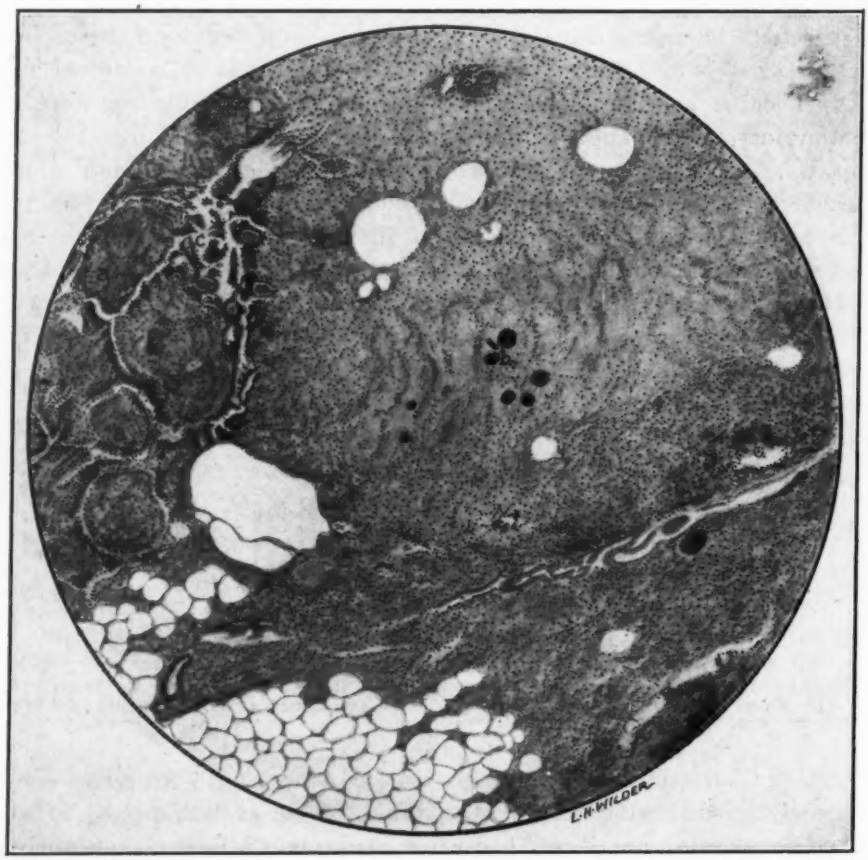

Fig. 2.—Omentum of rat infected with B. tuberculosis. Note the multinucleated eosinophilic cells forming syncytium-like areas (*a*), giant cells (*b*), and lymphocytic infiltration (*c*). 16 mm. objective, 10 eye piece.

that may be likened to a syncytium. In two weeks the proliferation of these multinucleated cells has extended to the spleen and liver. As the disease progresses these cells are very numerous in the omentum and spleen (Figs. 2 and 3), but never proliferate to any great extent in the liver (Fig. 4). In some instances a moderate degree of periportal cirrhosis may be seen as shown in Fig. 5. An occasional typical giant cell may be seen, and foci of lymphocytic infiltration is a constant occurrence in the omentum of moderately advanced infection. Proliferation of the eosinophilic multinucleated cells is also a marked feature in the lymph glands, as is shown in Fig. 6,

which represents a section of the substernal gland, taken one month after inoculation.

The pulmonary lesions are first observable histologically about one month after inoculation. The lesions are still very few at this time, but become quite numerous after sixty to ninety days following inoculation. Histologically the pulmonary tubercle is seen to consist of loosely packed aggregates of epithelioid cells, multinucleated cells resembling those seen in the lymphoid tissues referred to above, and occasionally true giant cells. Lymphocytes may be seen scattered through the "tubercle" or in discrete foci, with a tendency to arrange themselves peripherally around the large multinucleated cells. The epithelioid cells may be seen as distinct cells with

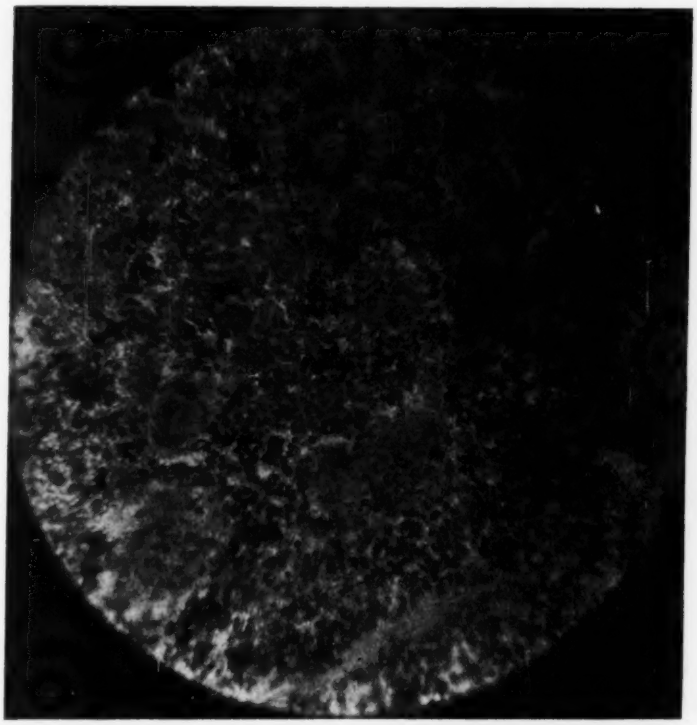

Fig. 3.—Spleen of rat infected with B. tuberculosis. Shows proliferation of multinucleated eosinophilic cells invading the Malpighian corpuscles and replacing the normal spleen pulp.

Photomicrograph, 4 mm. objective, 6.4 eye piece. Hematoxylin and eosin.

definite cell outlines or closely packed together, into what appears to be a single multinucleated cell. Carbolfuchsin stained sections of tissues that had been fixed in Zenker-formalin solution show the intracellular distribution of the tubercle bacillus in the pulmonary lesions, the bacilli being present in the epithelioid, multinucleated and the giant cells. These points are illustrated by Figs. 7 to 10. Sections of lungs of animals dying of the infection show enormous cellular proliferation, there being almost complete disappearance of alveolar spaces, and the cells are literally loaded with bacilli. (See Figs. 11 and 12.)

In hematoxylin-eosin sections of Zenker-formalin fixed tissues the cytoplasm of the epithelioid and multinucleated cells comprising the pulmonary "tubercle" often presents the appearance suggestive of minute fat globules. The lipoidal nature of these cells has now been abundantly verified by staining sections of frozen fresh or formalin fixed tissues with sudan III and osmic acid. Reference to Fig. 13 will clearly show that the cell aggregates, in which bacilli are found, have undergone certain fatty changes. Applica-

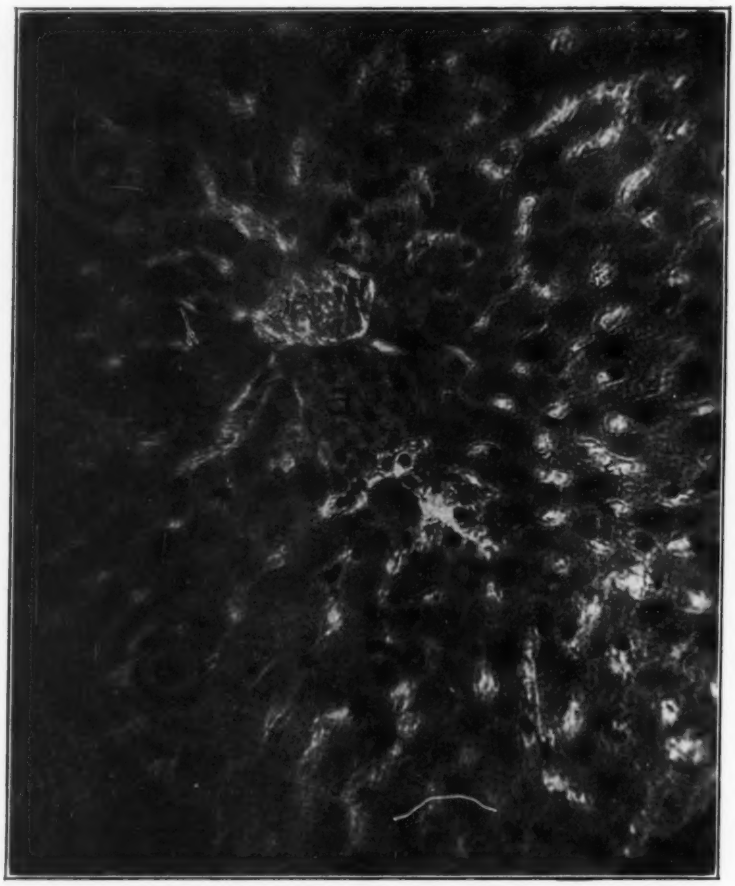

Fig. 4.—Photomicrograph of the liver of a rat infected with B. tuberculosis. Note the multinucleated cell (*a*) in relation to the sublobular vein and the bile duct. Hematoxylin and eosin, 16 mm. objective, 5 eye piece.

tion of osmic acid and sudan stains indicates that the fat is unsaturated, that it is intracellular, and finely granular.

This intracellular lipoid has been found in the pulmonary "tubercle" exclusively, whenever the latter has been subjected to examination, and the indications are that it is not influenced by changes in diet or by chemical agents, though further experiments on this point are in progress. There is no information at present available as to whether this lipoid is a product of the tubercle bacillus or of the altered metabolism of the cell within which the bacillus multiplies.

The multinucleated cells of other tissues of the infected rat, such as the spleen, liver, omentum, and glands do not undergo lipoidal transformation demonstrable by staining methods hitherto applied. It is perhaps significant that the multinucleated cells of these organs harbor relatively few bacilli, as may be judged from examination of carbolfuchsin stained sections of tissues fixed in Zenker-formalin.

Following the conception developed by Evans, Bowman and Winternitz,[11] Foot,[12, 13] and others of the endothelial origin of the epithelioid cell,

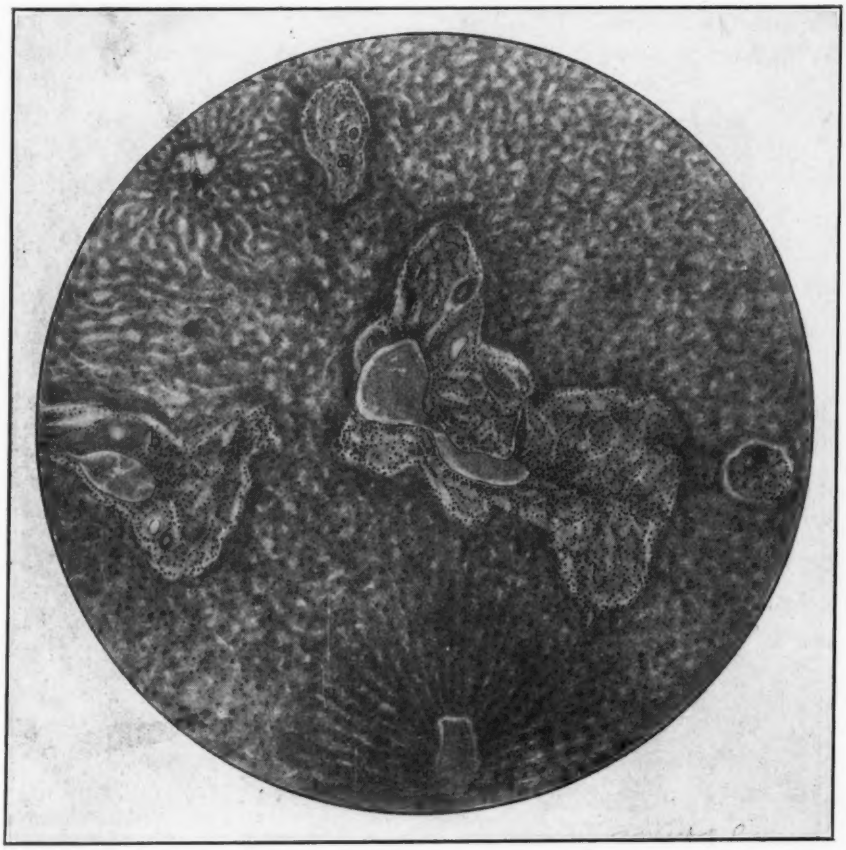

Fig. 5.—Liver of a rat infected with B. tuberculosis. Shows syncytial multinucleated eosinophilic cells within the liver lobule (*a*), in the portal canals (*b*), and some periportal cirrhosis. 16 mm. objective, 10 eye piece.

it seemed that it might be possible to influence the relatively simple cellular response to the tubercle bacillus in the rat by means of such agents as are known to be phagocyted by certain cells of endothelial origin. With this aim in view albino rats were treated with vital dyes and also inoculated with the tubercle bacillus. The dyes were administered either intravenously or intraperitoneally in doses of 5 to 10 mg. over considerable periods of time. Experiments with trypan-blue, which have yielded most interesting results thus far, clearly show that the vitally stained cell, probably the clasmatocyte, which according to the recent researches of Sabin, Doan and Cunningham[14]

is of endothelial origin, is quite distinct from the cell concerned with the reaction to the tubercle bacillus. In the spleen of the infected rat we find the large multinucleated syncytium-like eosinophilic cells, while in vitally stained nontuberculous animals there is evidence of much proliferation of megakaryocytes, and numerous clasmatocytes are found. The histologic picture of the lungs of vitally stained infected animals is most striking. The vitally stained clasmatocytes are seen in the interalveolar spaces, around the bronchioles, and in the neighborhood of the blood vessels. The epithelioid

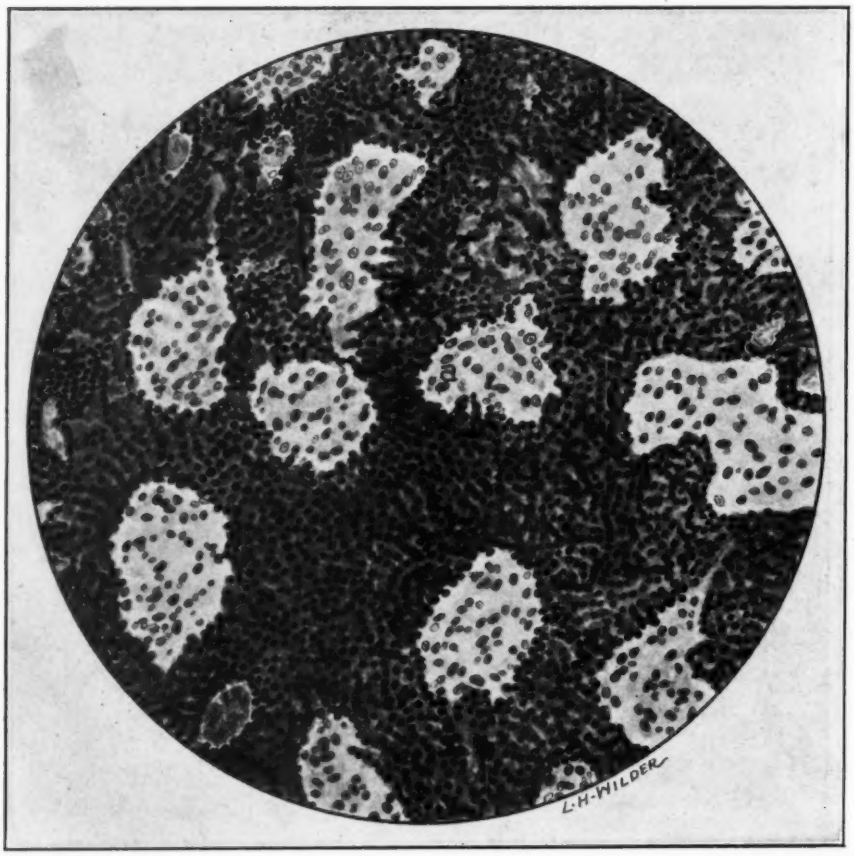

Fig. 6.—Substernal lymph gland of a rat after 30 days of inoculation with B. tuberculosis. Note the syncytial eosinophilic cells invading the lymphoid follicles. 4 mm. objective, 10 eye piece.

and multinucleated cells of the cellular aggregate, formed in response to tubercle infection, are not vitally stained. This can be most clearly demonstrated in frozen sections of fresh lungs of animals infected and vitally stained with trypan-blue, and later stained with sudan III. The cellular aggregate, constituting the pulmonary tubercle in the rat, stains more or less pink, depending on the amount of intracellular lipoid present, while the clasmatocytes are blue by contrast, showing a distribution as outlined above. By means of variously stained serial sections it can be shown clearly that the epithelioid and multinucleated cells, probably the monocytes of Sabin, Doan

and Cunningham,[14] contain the tubercle bacillus, and are chiefly concerned with the reaction thereto, while the vitally stained clasmatocyte is in no apparent relation to the tubercle. All attempts at modifying the tissue reaction of the rat to the tubercle bacillus by means of vital stains have failed to yield results, which is quite in harmony with the cited observation.

TUBERCULIN HYPERSENSITIVENESS IN RATS INFECTED WITH B. TUBERCULOSIS

This was studied in albino rats infected with 5 mg. of H 37 intraperitoneally and maintained on diets variously supplied with vitamin *A*. A single sample of a commercial old tuberculin was used in this series of experiments. The animals were tested at intervals ranging from 50 to 165 days following

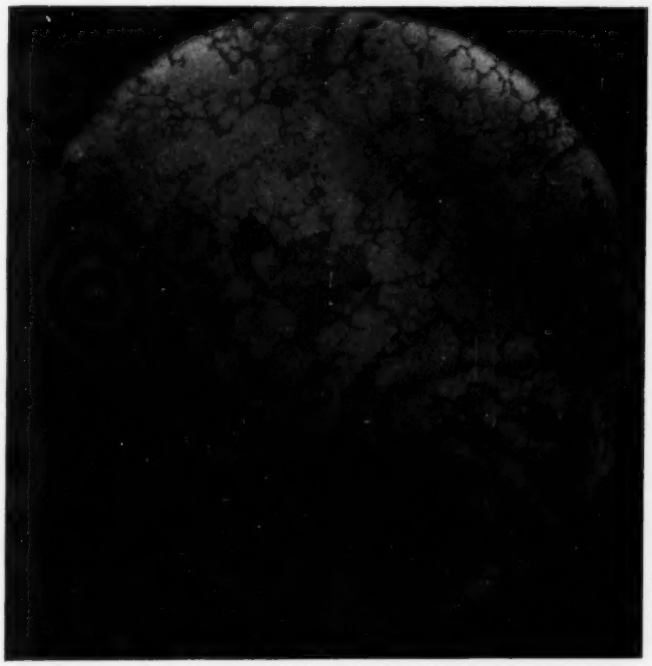

Fig. 7.—Photomicrograph of rat's lung after 60 days infection with H 37. Several cell aggregates constituting the pulmonary tubercle in the rat are shown. Hematoxylin and eosin, 4 mm. objective, 5 eye piece.

inoculation, the testing dose being 0.5 c.c. old tuberculin diluted with an equal volume of salt solution and injected intraperitoneally. Noninfected controls received like doses, and in some instances double the dose was given, without any effect, save for some slight symptoms, due to the irritant action of the glycerin which lasted for a few minutes. Many of the infected animals, on the other hand, became quite ill within a few hours of the injection, and many died within twenty-four hours. After the lapse of twenty-four to forty-eight hours the surviving animals usually appeared to have recovered completely.

The results of these experiments are given in Table I, which shows that the rat infected with the tubercle bacillus develops tuberculin hypersensi-

tiveness, and this is particularly apparent in animals on diets inadequate in vitamin *A*. With the exception of this deficiency, the diets were adequate in other respects. The protein in the diet of lots 56 to 61 (Table I) consisted of 40 per cent rolled oats supplemented with 10 per cent purified casein; 18 per cent purified muscle protein was the sole protein source in the diet of lots 38 and 39; and in the diet of the remaining lots 18 per cent purified casein furnished the protein moiety. Five per cent dried baker's yeast furnished the vitamin *B* requirement of the last two groups, while in the first group the animals derived this accessory from the oat kernel.

Fig. 8.—Higher magnification of one of the cell aggregates shown in Fig. 7. The cell aggregate consists of epithelioid cells (*a*), and multinucleated cells (*b*). Lymphocytic infiltration is seen throughout the cell aggregate, but more especially around the multinucleated cells. Hematoxylin and eosin, 4 mm. objective, 5 eye piece.

It is evident from the data given in the accompanying table that the adequately nourished tuberculous rat is relatively insusceptible to tuberculin shock. The precise nature of tuberculin shock is as yet unknown, but it may be of interest to point out that the rat is relatively insusceptible to other forms of shock, such as anaphylactic shock,[15] shock produced by diphtheria toxin,[16] and traumatic shock.[17] The recent work of Voegtlin and Dyer[17] on the enormous resistance of the rat to histamine and to the active principle of the infundibular lobe of the pituitary gland as well as to traumatic shock

TABLE I

EFFECT OF OLD TUBERCULIN ON TUBERCULOUS RATS MAINTAINED ON DIETS VARIOUSLY SUPPLIED WITH VITAMIN A. HALF C.C. O. T. DILUTED WITH AN EQUAL VOLUME OF SALT SOLUTION, INJECTED INTRAPERITONEALLY

VITAMIN A (BUTTER FAT) PER CENT	LOT	NUMBER OF ANIMALS	DAYS SINCE INOCULATION	WEIGHT IN GRAMS	NUMBER DYING	MORTALITY PER CENT
Infected with B. tuberculosis, H 37, 5 mg. intraperitoneally						
0	38	4	97		3	
	65	6	50		2	
	66	4	65	85–140	2	50
1	57	5	165		3	
	68	6	50	90–135	6	82
2	39	5	97		4	
	59	6	155		5	
	70	6	50	67–155	3	70
3	72	6	50	95–165	2	33
5	61	4	155	105–160	1	25
Noninfected controls						
0	64	5	—	97–125	0	0
1	56, 67	7	—	100–170	0	0
2	58, 69*	11	—	93–190	0	0
3	71	6	—	93–153	0	0
5	60	5	—	115–170	0	0

*Six animals of this group received 1.0 c.c. undiluted O.T.i.p.

throws much light on this important problem. These authors conclude from their work that the smooth muscle and the capillaries of this species react to shock-producing toxins in some peculiar manner different from those of other animals. Tuberculin shock may possibly belong to the same category, and the resistance of the rat to this form of shock might be explained on a similar basis. It may be added that from some work that is now in progress on pharmacologic studies of vitamin action, it is becoming quite evident that vitamin *A* deficiency affects in a specific manner the resistance of the white rat to certain poisons, and some evidence has been secured indicating that the vitamin *A* deficient rat shows an increased susceptibility to histamine in much the same manner as to tuberculin shock.

THE INFLUENCE OF VITAMIN DEFICIENCIES ON THE WHITE RAT EXPERIMENTALLY INFECTED WITH B. TUBERCULOSIS

In these experiments it was sought to determine the effect of diets deficient in either the fat soluble vitamin *A* or the water soluble *B* accessory on the course of tubercle infection in the rat. Animals approximately one month of age and weighing about 40 to 50 grams were selected from our breeding colony and separated into groups usually of six each. The animals of a given series were placed on a constant basal ration the food accessory under examination being added in varying and definite amounts to the basal ration. Each of the food mixtures was fed to each of two groups of animals of one sex and as nearly alike in age and weight as possible. After a week or two on the particular diet, the animals of one of the two groups were infected with H 37, as described in the earlier part of the paper, while those of the

other group were kept as controls. The feeding was continued, and, except for weekly weighings of the controls as well as of the infected animals, no further experimental procedures were applied. Histologic sections were made of the tissues of the infected rats that came to necropsy during the course of an experiment or at its termination. It was hoped that some information of value might be gained from a careful study of the tissue reaction and of the growth curve of the infected rat as compared with the noninfected control maintained on diets variously supplied with either vitamin *A* or *B*.

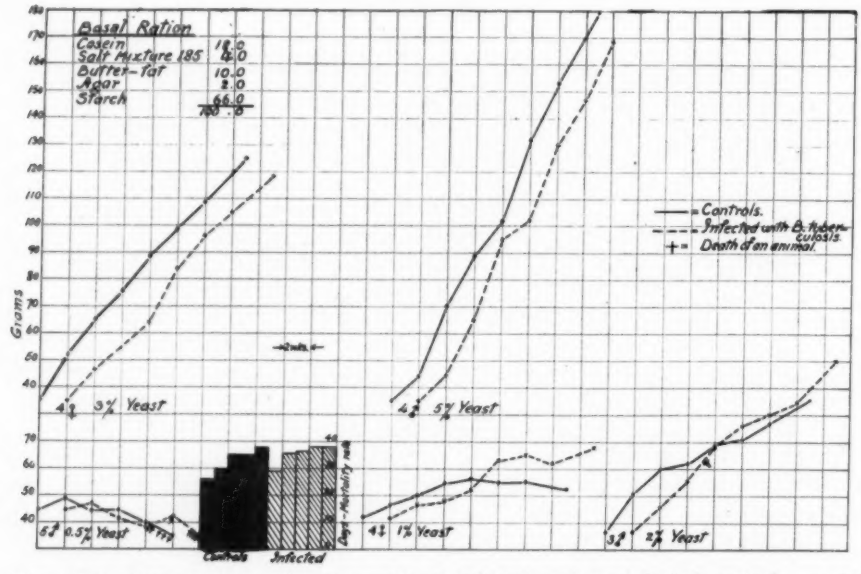

Chart III.—Comparative rate of growth of infected and noninfected controls on varying amounts of water-soluble vitamin *B*, added to a *B* free basal ration. The infected animal in no way differs from the noninfected control as regards its needs for the growth promoting water-soluble food accessory. The mortality rate of the two groups of animals on the *B*, deficient diet (0.5 per cent dried baker's yeast) is the same.

The experiments with the water soluble growth promoting and antineuritic factor were carried out according to the plan outlined above, using a basal ration of the following composition:

Casein (purified by the method of McCollum[18])	18.0
Salt mixture 185[19]	4.0
Clarified butter fat	10.0
Agar	2.0
Starch	66.0
	100.0

The water soluble food accessory was added in the form of dried baker's yeast in amounts ranging from 0.5 per cent to 5 per cent, replacing equivalent amounts of starch in the basal ration. The results, which are shown in Chart III, indicate that the rate of growth of the infected rat is not affected differently from that of the noninfected animal by vitamin *B* deficiency. The mortality rate was practically the same in the two groups which were fed a mixture containing a percentage of yeast below the minimal requirement for maintenance. The histologic findings of the infected animals of the several groups bore a relation to the length of infection, but there was nothing to

suggest an altered tissue reaction to the tubercle bacillus that could be ascribed to the dietary deficiency.

The observations made with the antixerophthalmic and growth promoting food accessory yielded results which were somewhat more encouraging. The earliest experiments conducted on this phase of the nutrition problem were made with a basal ration, slightly modified from that of McCollum, Simmonds, Becker and Shipley,[20] containing the following:

Rolled oats	40.0
Casein (purified)	10.0
Sodium chloride	1.0
Calcium carbonate	1.5
Olive oil	10.0
Starch	37.5
	100.0

Vitamin *A* was furnished in the form of clarified butter fat in amounts ranging up to 10 per cent, replacing equivalent amounts of olive oil. Inspection of Chart IV, summarizing the result of this experiment, will reveal that the infected animals fell distinctly behind the noninfected controls in the rate of growth on the lower allowances of butter fat, but made nearly as good a growth as the controls on a diet containing 10 per cent butter fat. At necropsy the usual degree of tuberculous involvement was found in the infected animals of this series, and histologic examination of the tissues failed to reveal anything different from the cellular reaction to the tubercle bacillus in the adequately nourished infected rat. These findings, which were reported in an earlier preliminary communication,[1b] were interpreted to mean that the rat infected with the tubercle bacillus requires more than the normal quota of vitamin *A* for its growth. Further observations on the relation of vitamin *A* to tubercle infection make the correctness of this interpretation somewhat doubtful, since it has not been possible to duplicate the results on a basal ration containing 18 per cent casein (for the composition of this see Chart I), supplemented with graded amounts of butter fat or cod-liver oil.

Examination of the weight curves of Chart IV makes it evident that the growth curves of the control animals are decidedly below normal, even on mixtures containing considerable butter fat. This becomes particularly clear upon examination of the individual weight curves of the animals of a group, when considerable variation in the rate of growth is noted. Growth of the rat is far more uniform and proceeds at a greater rate on a ration containing 18 per cent casein as its protein (see Chart I). The inferiority of oat-casein mixtures to an 18 per cent casein mixture in the nutrition of the rat was indeed pointed out several years ago by McCollum, Simmonds and Pitz.[21] At first sight it appeared that the phosphorus of the oat-casein mixture of Chart IV might have been somewhat low. A series of experiments was therefore carried out with the basal ration as shown in Chart IV with the addition of 1.5 per cent of monobasic sodium phosphate. The experiment was conducted for a period of eighteen weeks, at which time the average weight gains of the several groups were as follows:

BUTTER FAT P.C.	NONINFECTED	INFECTED
	Grams	Grams
1	45-108	43-91
2	30-116	30-77
5	25-130	25-96

The animals on this diet grew even more slowly than those of Chart IV. This was probably due to their younger age and smaller size when placed on the diet.

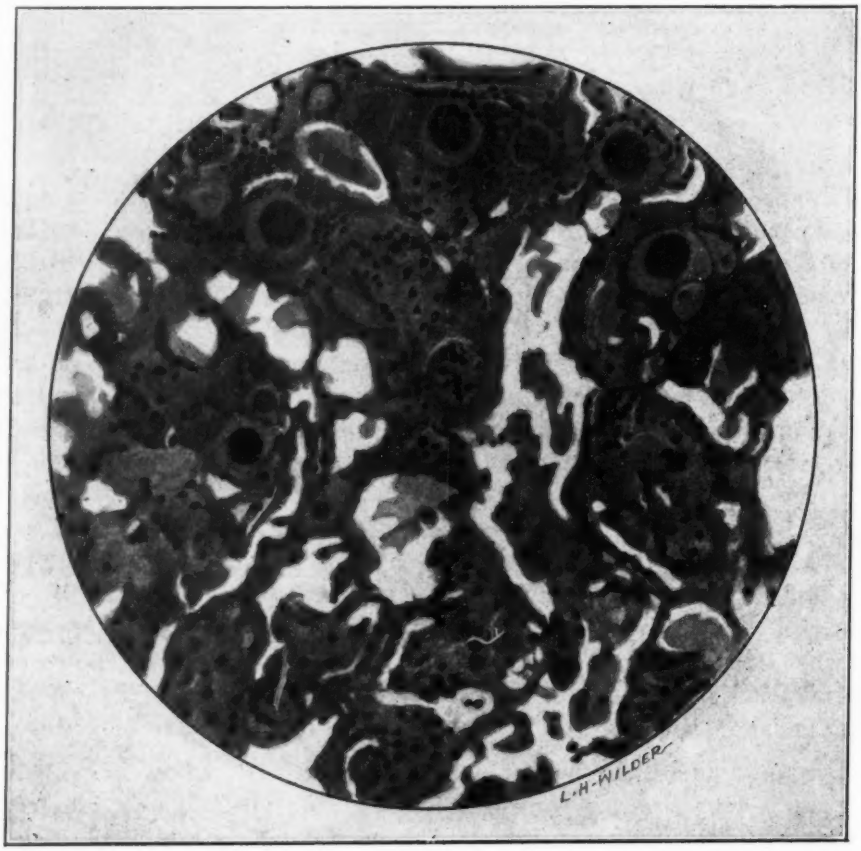

Fig. 9.—Drawing of 4 mm. objective and 10 eye piece magnification of rat's lung infected with B. tuberculosis 6 months. Epithelioid, multinucleated, and several typical giant cells are shown.

On further consideration it seemed probable that the deficiency of the oat-casein mixture might be due to inadequate supply of the water soluble vitamin. A third series of experiments were carried out with the same basal ration as shown in Chart IV with the addition of 2 per cent dried brewer's yeast. Considerable improvement in growth was effected by this addition. Whether this is due to the yeast protein or the water soluble vitamin cannot be stated at present. Here again as in the preceding experiments, the vitamin *A* deficiency was far more pronounced in the infected rat than in the noninfected control. The infected rats on the basal ration without butter fat (Group 111, Chart V) showed an extreme degree of malnutrition, character-

ized by loss of hair, marked emaciation, eczematous condition of the skin of the head and neck, besides the usual eye lesions. The animals of this group deteriorated rapidly, and most of them died within three to four months of the infection, while the controls, though showing some individual variation in the rate of growth, nevertheless presented a relatively fair state of nutrition. Fig. 14 shows the marked contrast in appearance of one of the infected animals (b) in group 111, and (a) a representative of the noninfected group

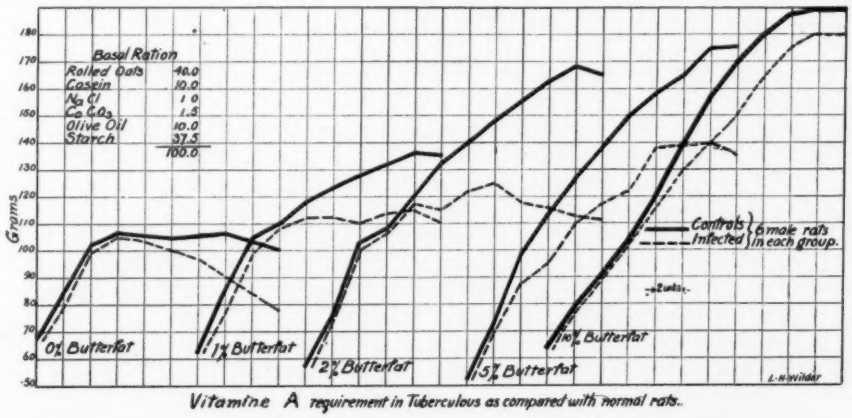

Chart IV.—Shows comparative rate of growth of infected and noninfected controls on varying amounts of vitamin *A* added to a basal ration nearly free from this vitamin. On a low allowance of vitamin *A* the growth rate of the infected rat is lower than that of the noninfected animal. With an increased allowance of butter fat the nutrition of the infected rat, as shown by the weight curve, is improved during its growth period and tends to approach that of the noninfected control.

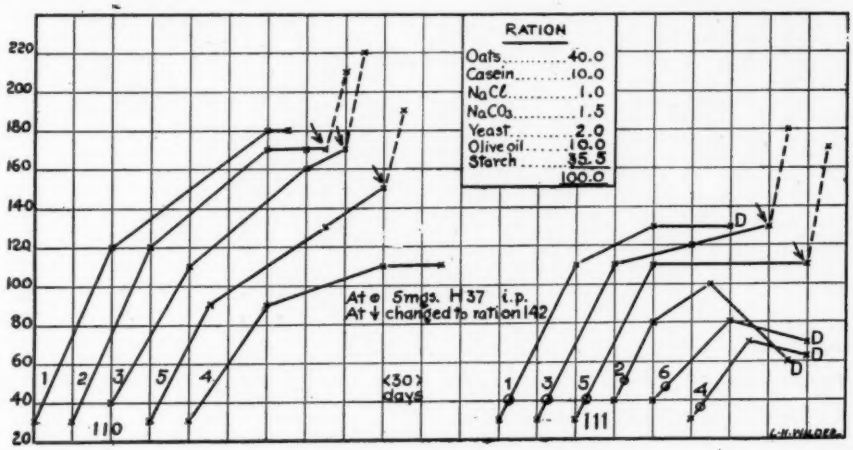

Chart V.—Course of tuberculous infection in rats maintained on a diet as of Chart II, but low in vitamin *A*. The infected animals (group 111) failed to grow anything like the controls, developed early cachexia and most of them died within three to six months after infection. At the point indicated by the arrow three of the controls and the two survivors of the infected group were put on a ration (similar to that of Chart I) adequate in all respects. Prompt resumption of growth occurred.

110, both of Chart V, maintained under identical dietary conditions. Rat C is included in the photograph to show the splendid state of nutrition of an infected animal on a food mixture including 10 per cent butter fat. The animals had been on the respective diets four months when the photograph was taken.

The results of the three series of experiments with the oat-casein food mixtures without butter fat or with the addition of a minimal amount thereof have consistently shown a much more marked degree of deterioration and decline in the case of the tubercle infected rat than in the noninfected control. The results furthermore indicate that with the inclusion of liberal amounts of butter fat in the oat-casein ration the differences in growth and in the general appearance of the infected and the noninfected animals tend to disappear during the growth period of the animal. It is not possible to state with certainty to what the early deterioration of the infected rat on the

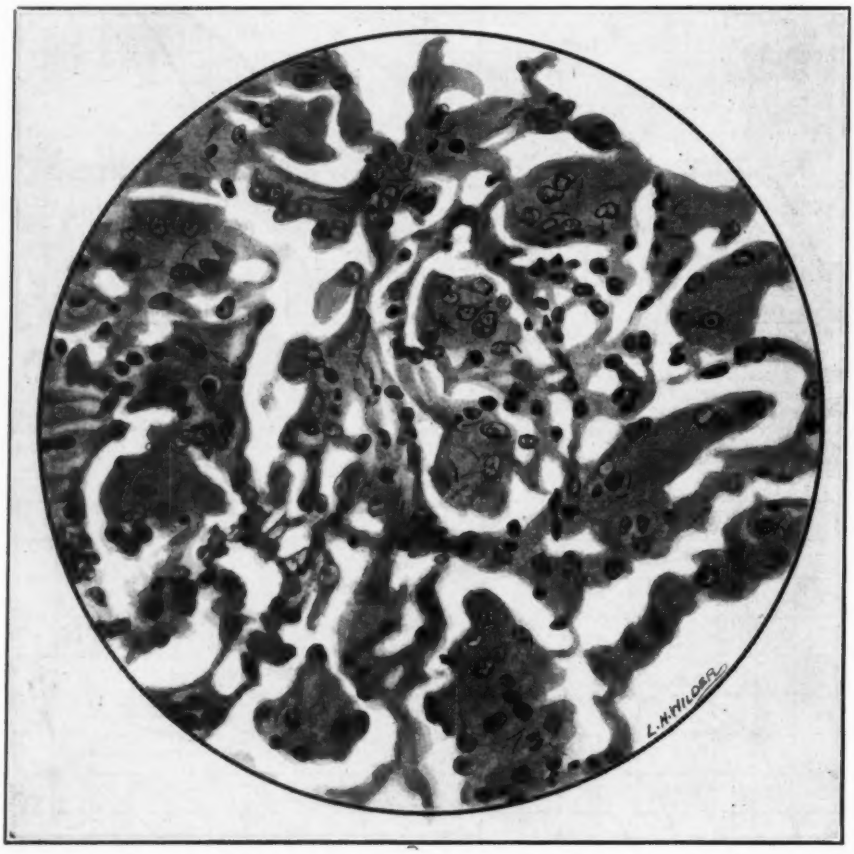

Fig. 10.—Section of lung as of preceding figure, stained with hematoxylin, carbolfuchsin and orange *G*. Shows many tubercle bacilli within the epithelioid and multinucleated cells. 1.9 mm. objective, oil immersion, 5 eye piece.

vitamin *A*-free oat-casein food mixture is due. While the symptoms are suggestive of an extreme degree of malnutrition, they are not unlike the cachexia of the terminal stages of tubercle infection in the adequately nourished rat, developing many months after inoculation. The observed facts appear to be susceptible of two interpretations. Either the tubercle bacillus multiplying in the tissues of the rat renders the latter more susceptible to vitamin *A* deficiency under certain dietary conditions; or vitamin *A* deficiency, in conjunction with a diet of low biologic value, decreases the resistance of the rat to tubercle infection to such a degree as to cause the appearance of grave

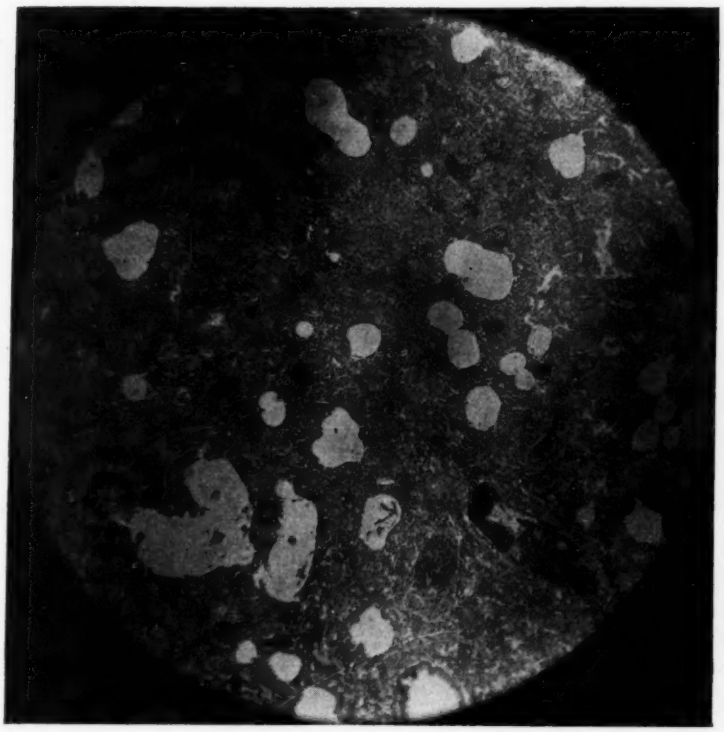

Fig. 11.—Photomicrograph of the lung of a rat that died from tuberculosis nine months after infection. The entire lung tissue is replaced with syncytial masses of multinucleated cells, nearly completely obliterating the air spaces.
Hematoxylin and eosin, 16 mm. objective, 5 eye piece.

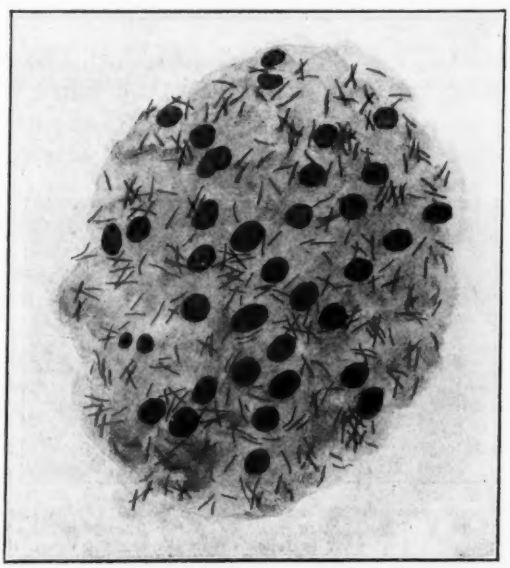

Fig. 12.—Drawing of a multinucleated cell from a section as shown in Fig. 11, stained with hematoxylin, carbolfuchsin, and orange G, and highly magnified. Note the enormous numbers of tubercle bacilli within the cell, which has not apparently undergone any degenerative changes. 1.9 mm. oil immersion objective, 5 eye piece.

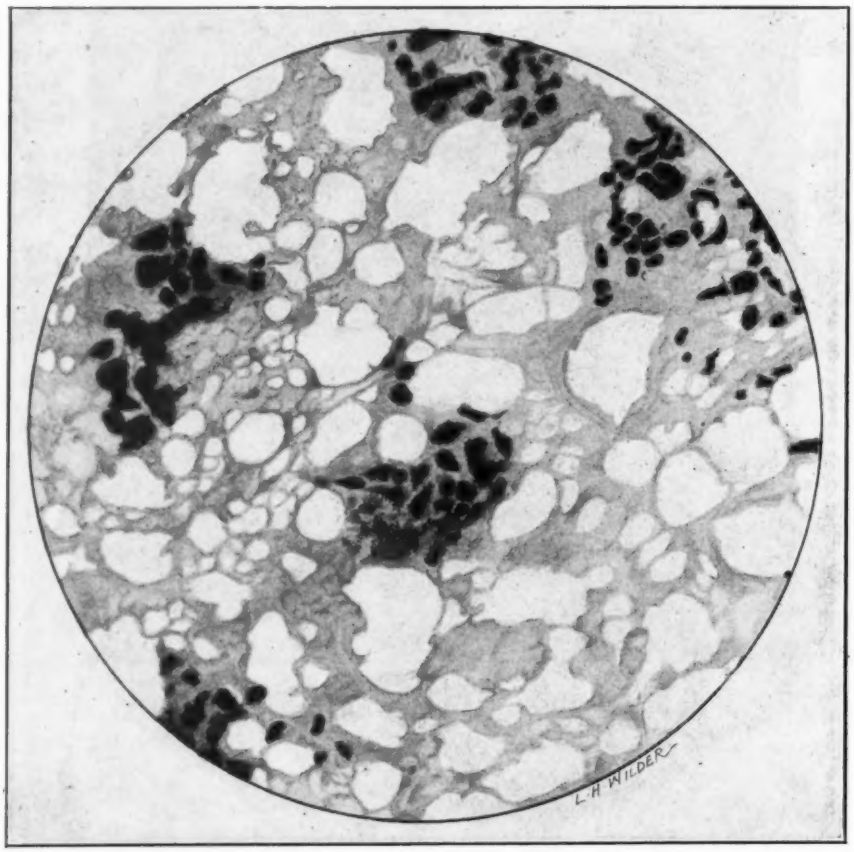

Fig. 13.—Drawing of a fresh frozen section of rat's lung infected with B. tuberculosis 5 months. The section which was stained with sudan III, shows the lipoid changes in the cell aggregate constituting the pulmonary tubercle in the rat. 16 mm. objective, 10 eye piece.

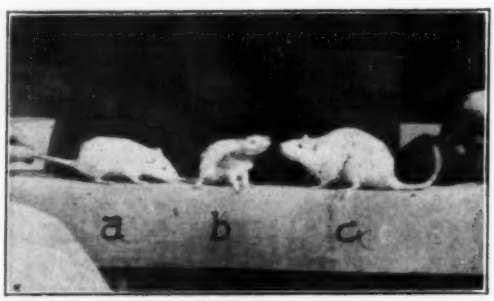

Fig. 14.—Photo of a representative rat of control group 110 (*a*), and one of the infected group 111 (*b*), both of Chart V. These rats have been for four months on a diet low in vitamin *A*. Rat (*c*) represents one of a group of infected animals having been the same length of time on a diet adequate in all respects.

symptoms in about one-third the time as compared with that required for their appearance in the adequately nourished animal. It should be noted that the increased susceptibility just referred to, whether it be an increased susceptibility to vitamin *A* deficiency, or a lowered resistance to tubercle infection in the *A*-deficient animal, has been successfully demonstrated only on the oat-casein mixture, a diet of relatively low biologic value. The relation of vitamin *A* deficiency to tubercle infection is not clearly apparent on a ration so constituted as to be adequate in all respects except the vitamin *A* factor. Thus, on the 18 per cent casein basal ration variously supplied with vitamin *A* in the form of butter fat or cod-liver oil, early nutritional failure in our experience is not apparent until practically all vitamin *A* is withheld, when the infected rat apparently deteriorates more rapidly and succumbs sooner than the noninfected one.

The precise rôle of other nutritional deficiencies in the relation of vitamin *A* deficiency to tubercle infection cannot be indicated at present. A more intimate knowledge of the nature of the oat-casein deficiency, or more properly the oat deficiency, would undoubtedly help in this matter. The work of McCollum, Simmonds and Pitz[21] on the dietary deficiencies of the oat kernel in the nutrition of the rat is helpful in understanding the observed differences in the behavior of the infected rat on the oat-casein basal ration on the one hand and on the 18 per cent casein ration on the other.

SUMMARY AND CONCLUSIONS

Experimental tuberculous infection in the albino rat is described.

It is shown that the albino rat, experimentally infected with B. tuberculosis, can be rendered susceptible to tuberculin shock by withholding fat soluble vitamin *A* from its diet.

Experiments are described indicating that the tubercle infected rat deteriorates more rapidly than the noninfected control when maintained on a diet of low biologic value and low in fat soluble *A*. Liberal allowance of fat soluble *A* appears to afford protection against the early deterioration. It is not certain whether this indicates a lowered resistance in the albino rat to tubercle infection or an increased demand for vitamin *A*. The increased susceptibility to tuberculin shock of the infected vitamin *A* deficient rat appears to offer evidence in favor of the former view.

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CARCINOMA OF THE STOMACH WITH HIGH BLOOD EOSINOPHILIA*

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EOSINOPHILIA, or the increase of eosinophilic polymorphonuclear leucocytes in the blood is observed under various circumstances, normal and pathologic. As a rather rare occurrence eosinophilia has been observed in connection with malignant tumors. Several authors have found a moderate eosinophilia (5 to 15 per cent) in cases of malignant tumors, but eosinophilia above 15 per cent has been found only in a few cases. Reinbach was the first to report a carcinoma of the neck with a high eosinophilia in the blood. The white blood count was 120,000 with 48.28 per cent eosinophiles. Three weeks later the white blood count went down to 52,000 with eosinophiles varying between 37.70 per cent and 46.15 per cent. According to Reinbach there are three factors which probably cause eosinophilia: (1) decrease in function of the blood-forming apparatus, (2) positive chemotaxis of toxins produced by the tumor cells, (3) disturbances in forming of white blood cells with a larger number of cells in circulation. Vosswinkel and Baradulin observed in cases where the tumors were removed a definite decrease of the acidophile cells. Kappis describes a case of carcinoma of the right lung with eosinophilia 33.6 per cent to 35.5 per cent. The histologic examination of the tumor tissue in this case showed strong infiltration of eosinophiles in necrotic areas. Many eosinophiles and eosinophile myelocytes were found in the tumor free bone marrow. According to Kappis, the necrotic tumor tissue exerts a chemotactic influence and causes a specific hyperproduction of eosinophiles and a secondary infiltration in the necrotic tumor tissue. Donati reports in his paper, among other cases, a carcinoma of the breast with 20 per cent eosinophiles; Dunger reports a carcinoma of the neck with 21,290 eosinophiles (38,330 leucocytes), with 21,400 eosinophiles (27,600 leucocytes), eight days later. Strisower described a carcinoma of the cervix with eosinophiles varying from 4.4 per cent to 45.37 per cent; Csaki, a malignant tumor of the colon with 30 per cent eosinophiles. Zappert, Oshima, Collins and Kaplan observed cases of sarcoma with high eosinophilia. (17.76 per cent to 31 per cent.)

The case which the writer wishes to report in this paper may be of considerable interest because of the fact that (1) it is the first reported case of carcinoma primary in the stomach with high eosinophilia; (2) there was opportunity to follow the case for a reasonably long time; (3) experimental work was done in connection with this case.

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Received for publication, August 9, 1925.

A. C., a female of forty years, came into the hospital April 7, 1925. Her menses began at the age of fifteen and were always regular. She had six children, three living and three who died very shortly after birth of childhood diseases. Since the birth of the last child, she complains about pain in the epigastrium. Otherwise she has never been sick. She lost twenty pounds in the past two years, five pounds in the last three months. The present complaints are chiefly localized in epigastrium. The pain in this region grows much worse when she eats. She vomits frequently, especially after large meals; even milk is vomited and curdled. The vomiting occurs usually half an hour following meals, at other times two hours after. Patient vomited blood twice in the past year. She has a good appetite; is very often constipated; is very anemic, and shows no disturbances of cardiorespiratory or of genitourinary apparatus.

The physical examination revealed apparent chronic illness, pallor and some emaciation. Eyes react to light, left eye does not respond to light as the right one. Tongue, teeth, mouth, neck, heart and lungs normal. Abdomen very soft and flaccid, with four distinct masses, palpable. (1) The largest and hardest mass was located about one and one-half inches below the costal margin, slightly to the left of the midline, in the epigastric region. (2) The second palpable mass was also hard and movable, located in the right hypochondrium; it was free from the liver, probably attached to the pylorus or gall bladder. (3) The third mass was hard and located in the left hypochondrium. (4) The fourth mass was in the umbilical region about one inch below the navel in the midline.

The x-ray report showed fluoroscopically a marked filling defect involving the entire pyloric region; it was spiculated and obstructive in its outline, typical of a neoplasm. Radiographic films conform to the above findings; some, six hour stomach stasis. Conclusion: characteristic evidence of advanced carcinoma.

Several laboratory tests were made. Blood examinations were made as shown in Table I.

TABLE I

DATE	RED BLOOD CELLS	WHITE BLOOD CELLS	HEMOGLOBIN	DIFFERENTIAL COUNT					
				LARGE AND SMALL LYMPHOCYTES	MONONUCLEAR AND TRANSITIONAL	POLYMORPHS			MYELOCYTES AND MYELOBLASTS
						NEUTROPHILE	EOSINOPHILE	BASOPHILE	
4–10 1925	4,240.000	16.300	70%	6.8	1.5	67.5	24.2	–	–
4–23	4,050.000	7.300	60	7.5	2.7	85.4	4.1	–	–
5–16	4,120.000	6.900	60	19.8	6.2	48.3	25.7	0.3	–
5–23	4,100.000	6.100	55	18.6	5.2	44.4	31.8	–	–

In the first differential count were found only a few megalo- and poikilocytes and a few micro- and normoblasts. In the following differential counts the number of immature red cells became gradually larger. Other laboratory tests were made as follows: Wassermann test was negative; 4 feces examinations for parasits were negative; 9 urine examinations showed a slight trace of albumin in a few instances, as a result of the increased number of pus cells.

Ten days after entrance into the hospital, the patient was operated upon. The lower half of the stomach was resected and the upper part, directly connected with the duodenum. The gross examination revealed a large tumorous mass occupying the lower half of the stomach, located chiefly on the posterior wall of the stomach and extending from the lesser curvature to the major and obstructing the pylorus. The surface of the tumor mass was soft and necrotic, the deeper parts were more resistant. No metastases could be detected in the abdominal organs. The microscopic examination of the tumor showed a typical adenocarcinoma, penetrating the mucosa, muscularis and

serosa. The connective and muscle tissue surrounding the carcinoma was to a great extent necrotic with no eosinophilic infiltration in this necrotic tissue.

EXPERIMENTS

The tumor mass was carefully cut out, ground and extracted with saline, which was added in the proportion 1:5. The flask was shaken and then put in the ice box for several days. The supernatant fluid was then injected intraperitoneally, intravenously, and intracardially in guinea pigs, rabbits and dogs, respectively. Complete blood counts were made on the animals previous to, one and three hours, and one, two and four days after the injections. The white blood cells showed a rise of two to three times as high as previous to injection after intracardial or intravenous injections. The leucocytosis disappeared after a few hours. Intraperitoneal injections showed a rise of white blood cells after two to three hours, usually disappearing completely within twelve hours. The differential count did not reveal in any of these injections an increase of eosinophiles; only the so-called pseudoeosinophiles in rabbits and guinea pigs showed a marked rise, while the dogs showed only a neutrophile leucocytosis.

In order to determine whether or not the blood of the patient contains toxic substances, derived from the tumor to cause eosinophilia, 2 c.c. of the patient's blood was defibrinated with an equal amount of a two per cent sodium citrate solution and injected in the ear vein of a dog. The white blood cells showed a 100 per cent increase three hours after injection. The differential counts revealed a high neutrophile leucocytosis. Not the slightest rise of eosinophiles could be observed. Forty-eight hours after the injection the white blood cells showed normal conditions.

To determine whether or not the eosinophiles possessed any phagocytic properties the defibrinated blood of the patient was washed three times with saline; after the supernatant fluid was removed, only the upper layers of the blood, which contained most of the leucocytes, were used for the study of phagocytosis. One mixture was prepared by mixing equal amounts of blood, typhoid bacilli and saline. The other mixture contained equal amounts of blood, typhoid bacilli and typhoid immune serum. Both mixtures were placed for half an hour in a water-bath at 37° C. After incubation, smears were prepared from both mixtures, stained with Wright's stain, and covered with Löffler's methylene blue for a few seconds. Eosin-hematoxylin and methylene blue proved to be as useful. No smear of either series showed eosinophile leucocytosis, while the neutrophiles were crowded with bacteria.

DISCUSSION AND SUMMARY

The writer presents a case of carcinoma with high eosinophilia in the blood. In the past only six cases of carcinoma with such high eosinophilia have been reported in the literature. In none of the previous cases was the tumor located in the stomach. In one of these cases, an eosinophilia was found in the surrounding necrotic tissue and a direct connection was assumed between the necrotic tumor tissue (toxins) and the eosinophile infiltration.

No experimental work was done to substantiate the above theoretic assumptions. In the case under discussion no eosinophilia was found in the tumor or surrounding tissue. Similar to the observations of Vosswinkel and Baradulin a decrease of eosinophiles after the removal of the tumor was observed. These two authors had no opportunity to observe their cases during a longer period following the operation. In our case a few weeks after the operation the eosinophiles showed again a rise exceeding the eosinophilia previous to the operation; whether this postoperative eosinophilia was caused through metastatic growth of the tumor, could not be determined. Neither the tumor extract, nor the blood of the patient could produce eosinophilia in animals (guinea pigs, rabbits and dogs). Our experimental work could not confirm the theoretic assumptions of Kappis, who thinks that the eosinophiles in the blood and in the tissues were caused by the toxins, derived from necrotic tumor tissue. Not the slightest phagocytic properties of the eosinophiles, even in the presence of immune serum, could be detected.

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A PLEA FOR A STANDARDIZED METHOD OF ESTIMATING AND REPORTING HEMOGLOBIN VALUES*

By Janvier W. Lindsay, M.D., E. Clarence Rice, M.D., and Maurice A. Selinger, M.D., Washington, D. C.

THE average routine blood examination usually includes an estimation of hemoglobin, and in the diagnosis of various types of anemias the clinician is in a great measure dependent upon a knowledge of the amount of this substance for a correct diagnosis.

Unfortunately for the general practitioner, a variety of instruments using different standards are on the market and are being used. Kern,[1] from the viewpoint of the internist, has shown how the variety of standards and the estimating of hemoglobin in per cent has served to make this examination of the least value in the routine blood examination. Many are not aware of the fact that each type of hemoglobinometer usually has a different value for its 100 per cent of hemoglobin. Chart I gives a curve setting forth the variety of standards of different observers, each representing 100 per cent.

If three different hematologists, each using a different type of hemoglobinometer, assuming each to be accurate, were to estimate the hemoglobin percentage of a patient, it would be difficult to convince the average attending physician that the following results were correct by the method used and that each represented the same amount of hemoglobin in grams per one hundred cubic centimeters:

Sahli-Leitz	100% = 15.00 gm. per 100 c.c.
Dare	109% = 15.00 gm. per 100 c.c.
Newcomer	88% = 15.00 gm. per 100 c.c.

A situation such as this frequently does arise when a patient is transferred from one hospital to another where different hemoglobinometers are used, with the result that complaint is often made that the hemoglobin estimation at one or both laboratories was incorrect. This misunderstanding could be overcome if the result was given in grams per 100 c.c. as well as per cent.

Many physicians have been in the habit of thinking of the amount of hemoglobin in an abstract way. One hundred per cent to them is equivalent to 5,000,000 erythrocytes per cubic millimeter, but they would ordinarily be at a loss to explain just what 100 per cent represents.

Inasmuch as it is well known that the hemoglobin content of the blood varies with the age, sex and time of day, it seems logical to allow considerable variation for the normal limits. Various observers (Dare, Haldane, Haden,

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists in Philadelphia, May 20 to 23, 1925.

From the Laboratories of Garfield Memorial Hospital, Washington, D. C.

Sahli, Williamson) have taken their normals from approximately 14.0 to 17.0 gm. per 100 c.c. and, in establishing an average, 15.6 gm. per 100 c.c. is generally accepted. The limits of 14.0 to 17.0 gm. per 100 c.c. is therefore suggested to be reported as the limits of normal when giving the hemoglobin content of the blood, the amount in grams per 100 c.c. as well as the percentage being reported as a routine. In the chemical examination of the blood a considerable leeway is allowed in the matter of normals, for example, sugar 80 to 120 milligrams per 100 c.c. of blood, and it is reasonable to assume similar variations for normal blood with reference to the hemoglobin content should be allowed.

The members of this Society would greatly clarify the general misunder-

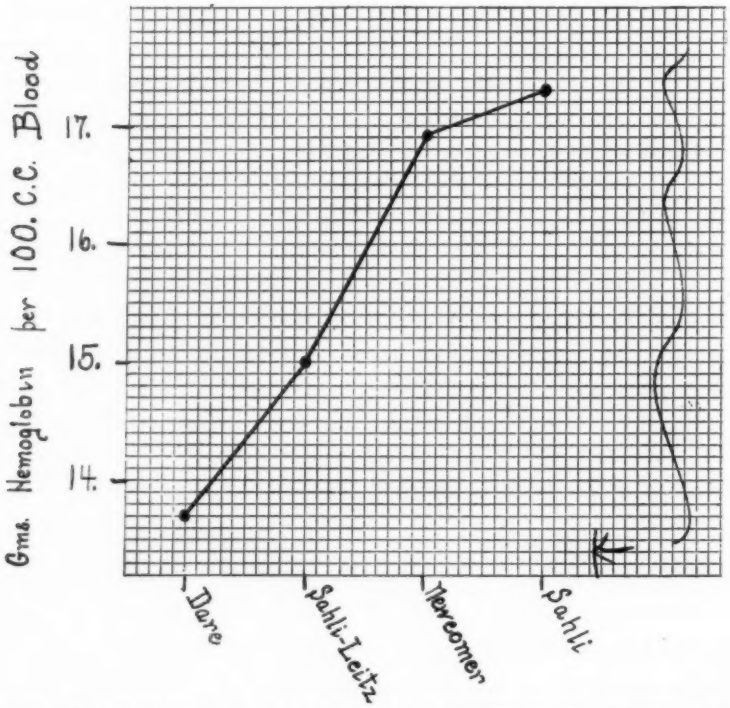

Chart I.—Variation in value of 100 per cent for various types of hemoglobinometer.

standing among clinicians if they would make it a practice to report hemoglobin values in the manner described.

As a result of the lack of proper standardization of instruments by the manufacturers or the inability to secure standardized replacements for certain other types, it is often impossible to give accurate results as determined directly without necessitating the use of a table of corrections for each instrument found to give inaccurate readings.

Comparison of the results obtained with the Sahli,[2] Dare,[3] and Newcomer[4, 5] hemoglobinometers with those obtained by Van Slyke's[6] oxygen capacity method and Wong's[7] colorimetric method was made (Table I) and showed such marked variations from the normal with the first two instruments as to make them useless for clinical work without restandardization.

TABLE I

COMPARISON OF ACCURACY OF VARIOUS TYPES OF HEMOGLOBINOMETERS

Instruments used were secured from various physicians' clinical laboratories except "factory Dare," which was obtained direct from the manufacturer

SPECIMEN	SAHLI	DARE			NEWCOMER	WONG	VAN SLYKE'S OXYGEN CAPACITY
		A	B	FACTORY			
1	18.7 (125%)	15.6 (114%)	14.4 (105%)	–	18.3 (108%)	18.8	18.78
2	18.9 (126%)	10.3 (75%)	10.4 (79%)	–	15.2 (90%)	15.79	15.89
3	15.0 (100%)	8.7 (63%)	9.2 (67%)	8.1 (59%)	9.1 (54%)	12.48	12.21
4	4.2 (28%)	2.0 (15%)	–	1.8 (13%)	2.9 (17%)	3.11	–
5	20.2 (135%)	10.7 (78%)	–	–	15.1 (90%)	14.35	–
6	15.7 (105%)	6.8 (50%)	–	–	9.1 (54%)	8.13	9.06
7	15.7 (105%)	7.0 (51%)	–	–	9.3 (55%)	7.89	7.88
Average Error	36.9%	23.7%	27.3%	38.0%	2.1%		

TABLE II

HEMOGLOBIN

WONG'S METHOD	VAN SLYKE'S OXYGEN CAPACITY METHOD	DIFFERENCE
18.80 gm. per 100 c.c.	18.78 gm. per 100 c.c.	+0.02
15.79 gm. per 100 c.c.	15.89 gm. per 100 c.c.	−0.10
15.87 gm. per 100 c.c.	15.16 gm. per 100 c.c.	+0.71
14.85 gm. per 100 c.c.	14.15 gm. per 100 c.c.	+0.70
12.48 gm. per 100 c.c.	12.21 gm. per 100 c.c.	+0.27
8.13 gm. per 100 c.c.	9.06 gm. per 100 c.c.	−0.93
7.89 gm. per 100 c.c.	7.88 gm. per 100 c.c.	+0.01
		Average +0.097 gm.

The instrument devised by Newcomer, using his glass plate standard, is in our experience the most accurate clinical hemoglobinometer which can safely be placed in the average clinician's hands. The parts of this instrument, so far as we have been able to determine, have been accurately standardized and can be replaced without introducing appreciable clinical error. This cannot be said of the Sahli, although a marked improvement in this instrument has been effected by the introduction of a solid glass standard in the place of the changeable liquid standard (Chart II). We have yet to examine a Dare hemoglobinometer giving sufficiently accurate results without being restandardized, and most physicians would not be apt to undertake this.

The physician who desires to standardize his hemoglobinometer need not possess the skill of a gas analyst, inasmuch as Wong's[7] method for determining the iron and hemoglobin content of the blood is sufficiently accurate as compared with Van Slyke's oxygen capacity method for practical work. This test requires not more than twenty minutes for its performance and it is comparable to Folin and Wu's test for estimating nonprotein nitrogen of the blood in simplicity (Table II).

The method of Cohen and Smith is excellent, but is impracticable for many small laboratories, whereas Newcomer's method is simpler and has been shown to be quite as accurate by Senty.[8] We believe that the Newcomer

hemoglobinometer is to be recommended as the instrument of choice in view of its (1) accuracy, (2) relative simplicity, (3) standardization in manufac-

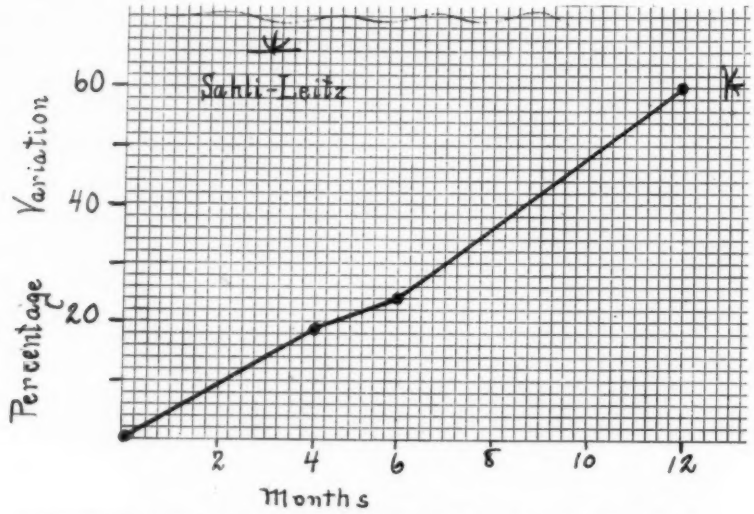

Chart II.—Change due to fading of Sahli standard color tube.

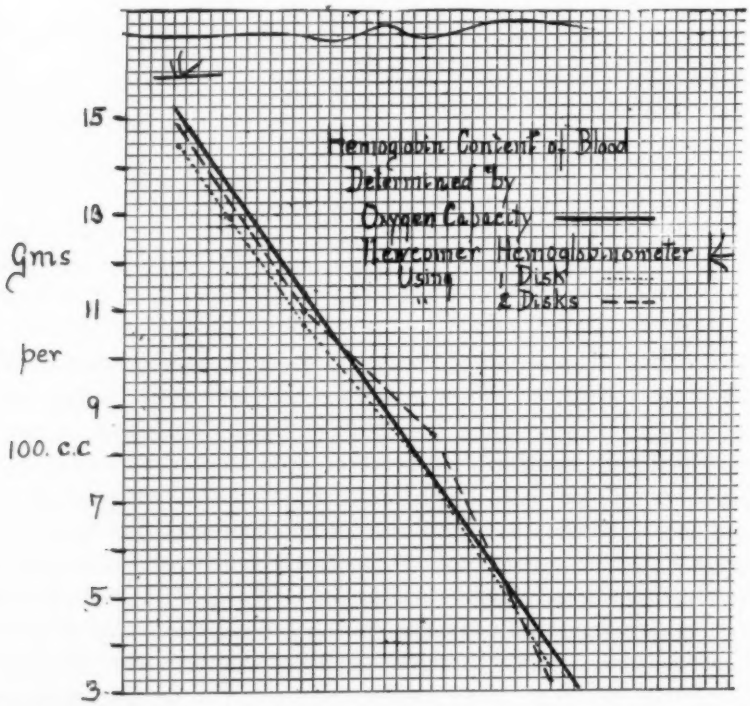

Chart III.—Hemoglobin determinations made with Newcomer hemoglobinometer using one and two color disks, as compared with results by Van Slyke's oxygen capacity method.

ture, (4) the general acceptance of the Duboscq type of colorimeter in colorimetric determinations, and (5) utilization of a color standard with absorption curve similar to that of acid hematin.

One of the objections to the Newcomer disk method has been the difficulty of matching colors, especially when the hemoglobin content of the blood was low. We believe that we have been able to simplify this matter by using two color disks of the same thickness for the standard and double the usual amount of blood (0.02 c.c.). For blood of hemoglobin content less than 6.77 gm. per 100 c.c. (40 per cent Newcomer), 0.02 c.c. of blood is drawn and used with only one of the glass plates as a standard (Chart III).

CONCLUSIONS

1. Few hemoglobinometers on the market are sufficiently accurate for clinical work.

2. Hematologists should make a practice of reporting the hemoglobin content of the blood in grams per 100 c.c. as well as per cent.

3. Fourteen to seventeen gm. per 100 c.c. is suggested as the normal limits of the hemoglobin content of the blood, these figures being used in the same manner as the normals in blood chemistry.

4. Wong's method of determining hemoglobin is sufficiently accurate for standardization of hemoglobinometers in the clinical laboratory.

5. The hemoglobinometer devised by Newcomer using a standardized glass color plate is recommended as the most accurate instrument for clinical work.

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DISCUSSION

Dr. Wm. H. Stoner.—It is common knowledge among laboratorians that there is more poor work done in hemoglobin estimations than in any other test. We checked up a series of approximately twelve Sahli hemoglobinometers that were in use. The Sahli pipettes vary and volumes vary from the calibrations by 15 per cent as a maximum. The dilution tubes vary as high as 10 per cent, and the color comparison tubes vary still more. Sahli is probably more widely used for determining hemoglobin outside the laboratory than any other test. With the advent of Dr. Pons into the Philadelphia Hospital this was looked into, and, through the normal process of evolution, he has arrived at almost the same conclusion as Dr. Rice. This alone is a recommendation for Dr. Rice's method. I think Dr. Rice may be complimented for coming to such a conclusion.

Dr. Carl L. Spohr.—I think we should not overlook the admirable work of Dr. Todd in his textbook on Clinical Pathology in reporting hemoglobin in grams, chiefly with the hope that later on this method might be adopted.

Dr. E. B. Krumbhaar.—When we first started some six months ago to report our hemoglobins in grams per 100 c.c., we were afraid that the clinicians on our staff would not like the method, but we have had no unfavorable comments. We feel that they, like ourselves, will soon become familiar with the reports in grams, so that, for instance, eleven grams of hemoglobin will represent as concrete a picture as, say, four million reds, and that it will not be necessary to transpose into an artificial percentage standard.

Dr. Pons has worked out for us what we feel is the best method of performing the test. It is to have the interne take to the wards a number of tubes of decinormal HCl solution, so that in each case the blood is mixed with the solution right in the ward. We know that a certain amount of time must elapse in order that the color may deepen to maximum capacity. By our method the solution has reached the maximum degree of color in the time it takes to get back to the laboratory, so that when the comparison is made in a standard colorimeter with the Newcomer amber glass plate the margin of error is reduced to a minimum. This test has been found to be foolproof and accurate. I would like to see this Society go on record as approving the method of reporting hemoglobin in grams per 100 c.c. instead of in some more or less arbitrary percentage.

Dr. John A. Kolmer.—I am going to ask the Committee on Laboratory Standardization of which Dr. Sondern is chairman to take these discussions to heart and consider them.

Dr. Frederic E. Sondern.—In going over the broad question of standard methods and in reading the many opinions you have been good enough to send to us, it is evident, as you know, that every laboratory worker has his favorite method which he believes is best for every laboratory procedure. At the same time I quite agree with the thought that committees might be organized to make studies of questions such as this and, while not necessarily establishing a standard for everyone to follow, to present such arguments as would make everyone glad to follow the suggestions. This would probably be justified if the opinion were that of the reporting workers and if their results were expressed in such a way as not to be open to any question. Relative to the matter of estimation of hemoglobin, it is my opinion that this is one of our most unsatisfactory determinations. I know that careful work with different apparatus gives different results and that the same apparatus under the same conditions among different laboratory workers will have readings which vary more than we desire. I wish I could make a recommendation to you which would obviate this.

Dr. Wm. G. Exton.—Dr. Rice certainly deserves to be complimented on his paper. The thought it brings to me is that this Society must take cognizance of the fact that not all of the apparatus and instruments offered by commercial houses are reliable, and that some time or other we should give our attention to finding means of approving those which are reliable. I have had considerable experience with commercial houses and all of them mean well but are apt to be misled. Some years ago we became interested in the various hemoglobin methods and found about the same condition of affairs as Dr. Rice describes. Owing to the fact that our specimens are not always fresh ones we have been depending meanwhile upon iron determinations, which are very simple to carry out.

Dr. C. A. Pons.—For the last year we have been using at the Philadelphia General Hospital, Newcomer's plate as our standard and have been reporting the results in grams. I feel that we are getting more consistent results with this method. If there is any error, I feel that we can put the blame on the person who collected the blood, since we do not have to contend with fading of the standard, precipitation, or inability to match the colors.

We have tried various types of colorimeters, and for our purpose Bausch & Lomb microcolorimeter 3625 has proved very satisfactory. As the normal average for an adult standard we have accepted Haden's standard 15.6 gm. per 100 c.c. of blood.

I believe that this is one side of the story; something has to be done to simplify hemocytometry. I have been very interested in Mr. Trenner's (of the A. H. Thomas Co.) work. He has made a pipette in which the dilutions are doubled and which probably will be very reliable and easy to manipulate. This automatic pipette is similar to the ones in use except that it has no graduations; the blood fills the capillary tube which ends where the bulb begins. Naturally capillary attraction ends here, and this is the correct amount of blood. Suction is necessary to dilute the blood up to the mark at the other end of the pipette. This gives the usual 1:200 dilution.

Dr. Rice (closing).—We have sent several of our color plates to the Bureau of Standards, for a permanent record of their spectral transmission. These will be of value in comparing with similar records made later, or if a new plate is secured, its graph can be compared with that of the original to determine whether it is a duplicate. This work was prompted by Dr. Kolmer's efforts to standardize the Wassermann reaction. I feel that there is often great error in this work and it is up to us to endeavor to eliminate it.

ANISOCYTOSIS AND INCREASED RED BLOOD CELL VOLUME WITH LITTLE OR NO ANEMIA*

By Mortimer Warren, M.D., Portland, Maine

PERSISTENCE of morphologic changes in the red blood cells is characteristic of pernicious anemia at all times during the course of the disease; less marked at the time of remission, these changes are none the less observable in smears. This finding ranks with achlorhydria as indispensable to the diagnosis of pernicious anemia. The determination of the hemoglobin as a presumptive test of the condition of the red blood cells may be misleading, whereas an examination of a properly prepared spread of blood permits immediate decision as to the necessity for further investigation of the patient so far as the blood-forming organs are concerned. With corroborative signs and symptoms, the diagnosis of pernicious anemia is easily established. Lacking this support, the significance of morphologic changes which approach those found in pernicious anemia is uncertain; I am speaking of variation in size of red blood cells with cells distinctly larger than normal, of variation in shape with cells of an oval form, and of cells which give the general impression of hyperchromia, a blood picture which is accompanied by a high volume and color index.

I have selected four cases of this type for presentation by means of microphotographs, using for comparison a case of pernicious anemia in full remission and a case of combined sclerosis with preponderance of cord changes and slight anemia.

METHODS

Hemoglobin by the Sahli method, standardized so that 100 per cent equals 5,000,000 normal red blood cells, occasionally checked by the Bausch and Lomb Newcomer method.

Platelets by the Olaf Thomsen method as described by Gram.[1]

In computing the volume index, the method of Haden[2] was used.

The charts are self-explanatory except that the notation "Poly." means the percentage of polymorphonuclears, and the notation "4" means the percentage of polynuclears having four or more lobes.

Chart I (555/25).—A man of fifty-five years who had been ill for two years at the time of this examination. This is the second remission. The course, history, and signs are typical and require no comment.

Chart II (262/25).—A man of forty-five years, referred by Dr. Henry Swift for lumbar puncture because of absent knee jerks and ataxia. He had some numbness of the legs and, to a lesser extent, of the hands, impairment of position sense, and inability to use his legs without support. The pupils reacted to light. The spinal fluid was clear with one cell per cubic millimeter; a trace of globulin; no curve with gold solution; and a negative Wassermann. These symptoms began two years earlier, following mumps.

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20 to 23, 1925.

CHART III (378/25).—A nurse thirty-four years of age, who has not regained her previous health since having influenza in France in 1917. She looks anemic, tires easily, has considerable flatulence with no abdominal pain, at times legs feel numb with no changes in reflexes, has apparent pallor without icteric tint, tongue is normal.

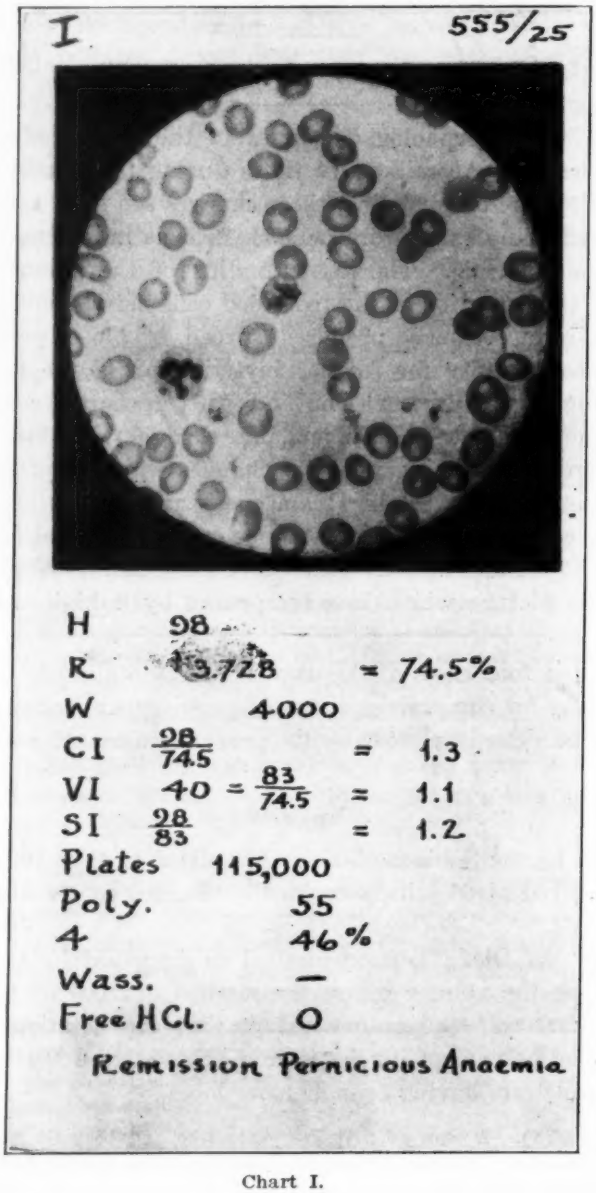


Chart I.

CHART IV (665/25).—A man of thirty whose complaint was of tiring easily. He was told he had malaria in 1917 in North Carolina. Reflexes normal; station normal; tongue negative; joints feel a little stiff. Gonorrhea four years ago. Wassermann strongly positive.

CHART V (753/25).—A woman of twenty-five, patient of Dr. E. W. Gehring, who complained of nausea, weakness, and tiring easily. No physical signs. Basal metabolism (−30). Blood pressure $\frac{110}{75}$. No paresthesia. The characteristic physical and mental features of myxedema were absent.

CHART VI (795/25).—A man of thirty, a clinic case of cerebrospinal lues with strongly positive Wassermann in the spinal fluid, a paretic curve, a cell count of twenty, decided trace of globulin. The notation of negative Wassermann on this chart is not representative of the usual state of his blood; under treatment, the Wassermann was reduced, but has since recurred. He has absent knee jerks, stiff pupils, slight unsteadiness, numbness and lightning pains in his legs.

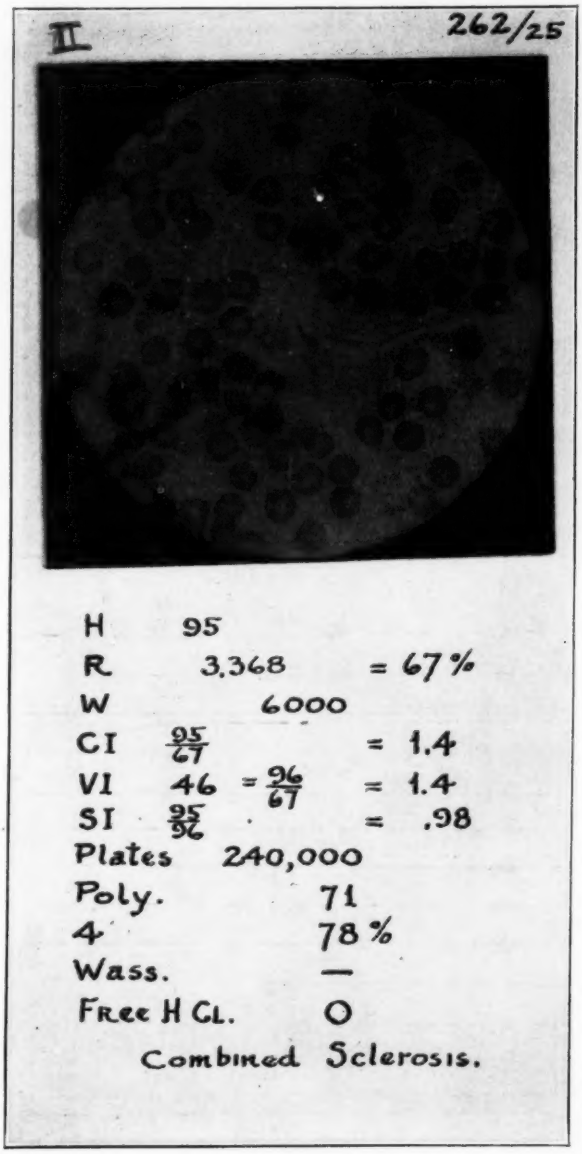


Chart II.

DISCUSSION

In Chart II is represented a type of case with disabling cord lesions and no apparent anemia, to which Woltman[3] calls attention. The blood morphology is sufficiently distinctive to allow of the diagnosis of pernicious anemia.

In fact, the diagnosis rests on the blood picture if all cases of sclerosis are accompanied by achylia gastrica, as found in those reported by Vanderhoof.

The other charts represent dissimilar clinical types, in common having ill-defined disturbance of general health, which in two cases can be attributed

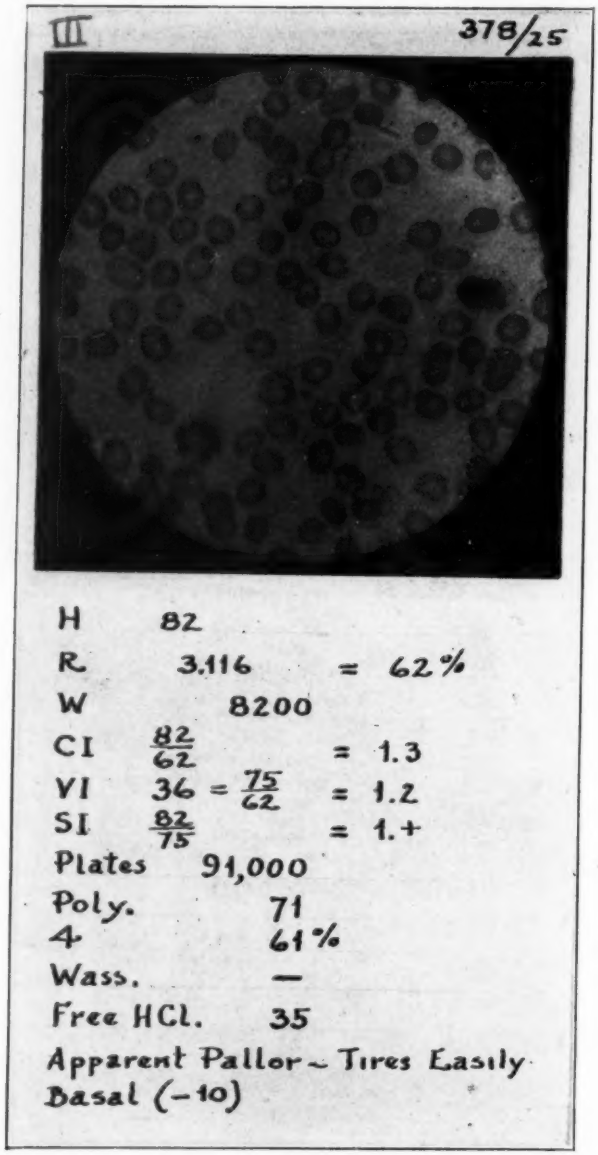


Chart III.

to the constitutional effect of syphilis. These blood findings are suggestive, but can be interpreted with relation to pernicious anemia only in a speculative sense.

According to Hurst and Bell[5] achlorhydria precedes the anemia; may there not be as well a constitutional susceptibility on the part of the marrow

in certain individuals, which manifests itself by changes in the blood in various disturbances of health without the presence of other factors necessary for the development of the completed picture of pernicious anemia?

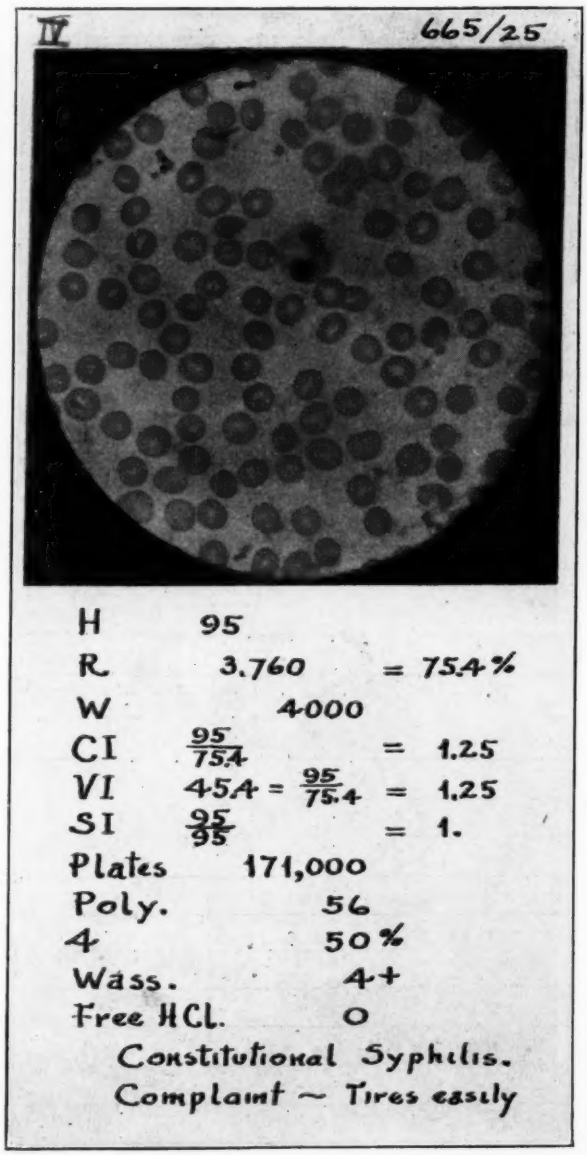
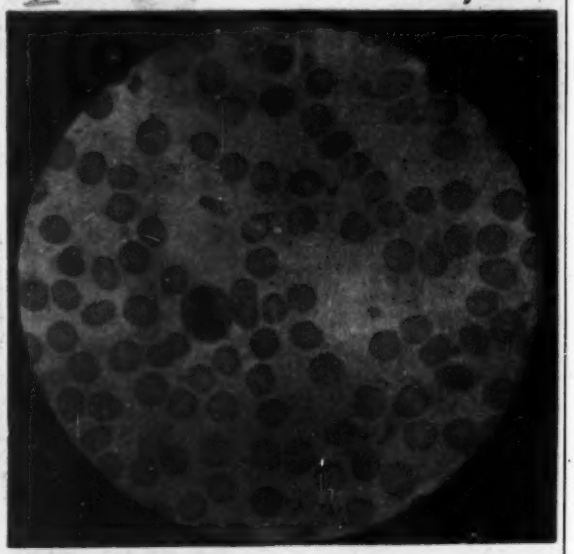


Chart. IV.

SUMMARY

These charts have been shown for the purpose of demonstrating minor changes in blood morphology which simulate those characteristic of pernicious anemia in cases in which the diagnosis of this condition is not confirmable by other data and in which the anemia is minimal.

V 753/25

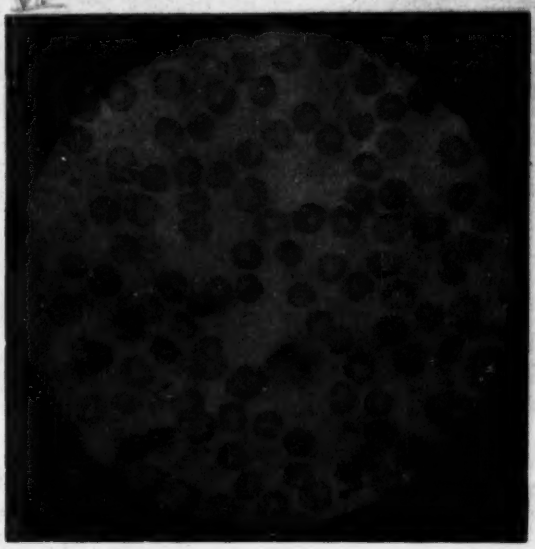


H 78
R 3.088 = 62%
W 4000
CI $\frac{78}{62}$ = 1.25
VI 33.3 $\frac{69}{62}$ = 1.1
SI $\frac{78}{69}$ = 1.1
Plates 236,000
Poly. 51
4 58%
Wass —
Free HCl. 15" = 0 1 hr. = 10

Basal (-30)

Chart V.

VI 795/25



H 80
R 3.728 = 74.5%
W 4000
CI $\frac{80}{74.5}$ = 1.+
VI 45.7 $\frac{95}{745}$ = 1.27
SI $\frac{80}{95}$ = 0.84
Plates 222,000
Poly. 68
4 40%
Wass. —
Free HCl. 25
Old Lues ~ Cerebro Spinal.

Chart VI.

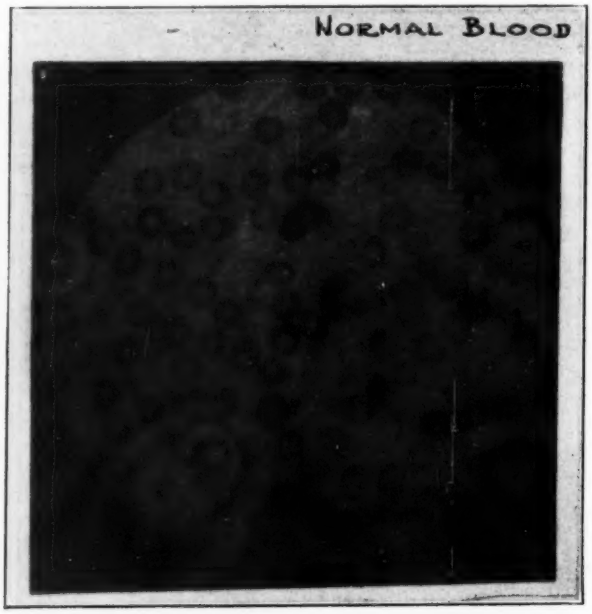


Chart VII.

In conclusion, thanks are due to Harold E. Ayer, B.S., for his assistance in photomicrographic work, and in the preparation of the charts.

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DISCUSSION

Dr. Frank W. Hartman.—We are very much interested in the case mentioned by Dr. Warren showing a minus 30 basal metabolic rate. We have followed some cases of myxedema, doing volume index determinations, and we find them usually above one. Could it be that the case just cited is a myxedema rather than a primary anemia?

Dr. H. A. Heise.—I enjoyed this paper very much. I must, however, add a little criticism. I see many articles written for journals in which blood counts using three or more significant figures are given. Such accuracy is not only improbable, but of no clinical value.

Dr. Mortimer Warren (closing).—In computation of the volume index and color index, carrying out the figures to the second decimal point as an evidence of accuracy is open to criticism. The figures as obtained were used, and in case the index figured 1.26, for example, such a figure was used rather than 1.3 or 1.2+, which I believe would have been more fitting. The case to which Dr. Hartman refers may be one of myxedema. I feel uncertain about the significance of the blood changes.

BRONCHIAL SPIROCHETOSIS (CASTELLANI)*

By Thomas L. Ramsey,† Toledo, Ohio

THE fact has been known for some time that an organism resembling the spirillum of Vincent can be and is the cause of certain types of bronchial and pulmonary infections, nevertheless, it seems that the medical profession at large is somewhat tardy in recognizing such a condition.

It is the object of this paper to summarize the many cases reported and to add an additional seven cases that the writer has himself observed.

1866. Jaffe and Leyden[1] in Holland described the finding of spirochetes in the sputum in several cases of pulmonary gangrene. This work was not generally known until

1906. Castellani,[2] working in Ceylon, published a report of two cases of pulmonary invasion by an organism he called and classified as a spirochete. He reported these cases as bronchial spirochetosis. The symptoms in brief were cough, fever, pain in the chest, with or without hemoptysis. The other findings did not resemble tuberculosis. The presence of numerous actively motile spirochetes in the sputum and the absence of tubercle bacilli gave him the impression that the spirochetes were the cause of the infection. The same year Branch,[3] in the West Indies, confirmed these findings.

1909. Waters[4] in India, Johnson,[5] Phalen and Kilbourne[6] in their report to Washington, D. C., told of similar cases.

1910. Rothwell[7] reported four cases of what he termed Vincent's angina, this being the first mention of any cases in the United States.

1911. Chamberlain[8] reported several cases occurring in the Philippine Islands.

1913. Chalmers and O'Farrel[9] reported the disease in Africa.

1914. Taylor[10] and Harper[11] reported cases in the tropics. At this time the writer reported two cases in Santa Marta, Colombia, S. A.

1915. Fantham[12] published a complete work on this subject. Lurie[13] reported a case in Serbia, Thompson[14] several cases in Salonica, and Galli-Valerio[15] several cases in Switzerland.

1918. Voille,[16] in France, observed many cases in natives coming in from the east as well as in several Europeans.

1919. Farah[17] described cases in Egypt.

1920. Nolf[18] reported cases in Belgium; Lewis,[19] in Cuba; Mason,[20] in the United States; and Trocello,[21] in Italy, reported cases of this infection.

1921. Levy[22] reported two cases in Texas; Bloedorn and Houghton[23] reported cases seen in the U. S. Naval Service. Kline[24] reported three cases of pulmonary gangrene with similar findings in the sputum and in micro-

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, May 20 to 23, 1925.

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scopic sections from the tissues obtained at autopsy. Segura and Puccio,[25] in Buenos Aires, reported cultivation of Castellani's spirochetes from the sputum of a patient suffering from an acute pulmonary condition.

1922. Faust[26] reported cases of bronchial spirochetosis in China and stated that a certain proportion of these cases have pulmonary tuberculosis as a primary infection and a secondary spirochetosis. Other cases show simply spirochetosis, usually of the chronic type, although cases of acute infection were observed. Alfaro[27] in Argentina reported four cases of spirochetal infection resembling tuberculosis, one of which had involved the lung. Engelsen[28] in Christiania reported a case of three months' duration, which had previously been diagnosed as tuberculosis, until the spirochetes were found in the sputum. Under arsenical medication the recovery was soon complete.

1923. Pageniez and Ravena,[29] France reported a case of fourteen years' duration occurring in a young woman. Numerous spirochetes were found in the sputum, no clinical or radiologic changes were detected in the lungs, also in this case the arsenicals were without effect. Carulla, Besimsky and Sanguinetti in Argentina[30] reported a case of four years' duration with symptoms of chronic bronchitis before the sputum became hemorrhagic and Castellani's spirochetes were discovered. Risquez, in Caracas, Ven.,[31] reported a series of cases occurring in the course of a routine examination of two hundred patients, finding 29 per cent positive with spirochetes. He states that the disease seems to be contagious and reported antimony by the intravenous injection as the most effective treatment.

1924. Maglione and Palazzo[32] in the Argentina reported a case from which they were successful in transmitting the infection to guinea pigs. The disease proved to be contagious, rapidly spreading to other pigs. Gazette and Massias[33] in Spain reported several cases, one case in particular which did not respond to any treatment. A fusiform bacillus was present, associated with the spirochetes, in all of their cases. Jackson, U. S. A.,[34] reported a case with pseudomembranous bronchitis due to Vincent's organisms. McNeill, U. S. A.,[35] reported a case with infection of the parenchyma of the lung, indistinguishable from tuberculosis, which recovered rapidly under intravenous arsphenamine therapy. Schwarz[36] reported in the London *Lancet* six cases with typical symptoms but always associated with pyorrhea alveolaris and very few pulmonary signs. All cases recovered under arsenical treatment.

Several other writers have observed infections of this nature, including J. H. and W. B. Rothwell and W. H. Peters. From this long list one can readily observe that this is not a fanciful condition arising in the imagination of any one mind, and certainly it deserves more careful consideration than has been awarded it in the past.

All writers agree upon the appearance of the organism and in their opinion that the spirochetes are capable of producing bronchopulmonary lesions. Many believe, however, that they are a species of the ordinary spirillum found in the mouth that are capable of assuming pathogenic properties. Some think them the same as Spirocheta micro- and macrodentium; others as

a form of Vincent's spirillum as they are often associated in the lesions with a fusiform bacillus.

Castellani classifies them as a spirochete or spironema rather than a treponema. They are very actively motile, ends are accuminate; they are highly refractile, and vary in length from 5 to 25 microns and in breadth from 0.2 to 0.3 microns. Their motility is soon lost after removal from the body, when granules are formed, from which it is believed new spirochetes develop. The undulations vary in number from 2 to 16, the length of each being from 1 to 1.9 microns. The organism has no spores or flagellae and no undulating membrane. It stains poorly with ordinary analine dyes; it is gram-negative; it stains best by Fontana or Levaditi's stain; and it is fairly well stained by steaming with carbolfuchsin.

The organisms are difficult to cultivate and ordinary laboratory animals most often die from other infections which are transmitted along with the spirochetes, owing to the inability to separate the organisms. The disease has been reproduced in the monkey; it has also been successfully transmitted to guinea pigs.

SYMPTOMATOLOGY OF THE INFECTION

The Onset varies considerably; the majority of the cases reported were at first diagnosed as tuberculosis, and many had the classical symptoms of this disease such as fever, cough, profuse expectoration, with or without hemoptysis, night sweats, loss of weight, headaches, etc. Other cases had no appreciable symptoms except the spitting of blood. The chest findings also vary considerably from slight loss of resonance and diminished breath sounds to complete dullness over whole lobes.

The Sputum Findings are quite similar in all cases, the absence of the tubercle bacilli and elastic tissue and the presence of numerous short and long spiral organisms together with the ordinary bacteria of the sputum. Often an association with large numbers of fusiform bacilli is noted. This organism is often found in the normal sputum and its presence noted in many conditions. Many think it has nothing to do with the infection and is present only as a secondary invader, as are the other mouth organisms. Others place considerable importance upon these bacilli, stating that as in Vincent's angina they are but transition forms of the spirillum. There have been cases reported where the severity of the infection has been such that pulmonary gangrene resulted and death occurred. The organisms were found in large numbers in the tissues at autopsy.

Other findings, such as secondary associated anemia and occasionally gastric disturbances due, in the writer's opinion, to the swallowing of large numbers of these organisms during the attack. The teeth and tonsils are occasionally involved.

The course of the disease is usually acute, and hemoptysis is a frequent symptom. The disease may be self-limited running a course of ten days or two weeks, with a return to normal and a recurrence after a brief period. The tendency is to become chronic when the symptoms may become quiescent, the spirochetes remaining in the sputum. The lung involvement appar-

ently varies considerably, and there may be nothing more than bronchitis or a peribronchial infiltration. The areas may increase in size until lobules and even whole lobes may be involved. Pulmonary gangrene may occur.

In laboratory findings the sputum examination is by far the most important. It is necessary to use a stain that will bring out these organisms, as otherwise they are easily overlooked. The persistent absence of the tubercle bacilli and elastic tissue and the finding of numerous spiral organisms together with the ordinary bacteria of the sputum, will generally suffice to call one's attention to the possibilities of spirochetosis. An associated anemia with a fall in the number of red blood cells and hemoglobin is usually present, especially where hemoptysis takes place. The white count is usually normal or slightly elevated with an increase in the large lymphocytes and eosinophiles. Urinary findings usually negative; blood cultures persistently negative.

Treatment.—The disease is readily amenable to the proper treatment. Expectorants seem to aggravate the condition, while the arsenical compounds act almost as a specific. Such combinations as Fowler's solution with hypophosphites followed by iron and quinine will work wonders in these cases. The use of neosalvarsan intravenously is also indicated. Other symptomatic treatment, especially where considerable hemoptysis occurs, may be recommended. Antimony has been recommended, especially by intravenous injection.

Conclusions.—These cases are not common but undoubtedly they are more common than we have heretofore believed. Many think this infection is present only in the tropics or in persons coming from these regions. If more examinations were made with this diagnosis in view, however, we would find that some of our supposed tuberculous cases would undoubtedly be bronchial spirochetosis.

CASE REPORTS

I have to report, besides the two cases I saw in Colombia, S. A., three cases that I have seen in Toledo during the last seven years and two others that I did not see but of which I made the sputum examinations, and, in reviewing their histories, I believe them to be of this nature.

Two cases reported by the writer were observed in the hospital of the United Fruit Company, Santa Marta, Colombia, S. A., 1914. These were reported by letter to the medical department of the company, enclosing paper on bronchial spirochetosis for publication in the London *Lancet*.

CASE 1.—Adult male, Colombian, apparent age thirty years. Family history negative.

Past History.—Stated that he had never been sick, not even had he suffered from malaria, so prevalent in that region.

Present Illness.—Two months previous to entering the hospital he had been exposed to several severe wettings from tropical rains and had slept out in the open; he had contracted a cold on the chest and a slight cough. This continued without fever for about ten days, when he had a chill and was sent to the hospital for treatment. Diagnosis, tuberculosis. Complains now of headache, fever, chills and cough with profuse expectoration of slimy sputum. No blood in sputum.

Examination showed *head* normal; some sore throat and injection of tonsils; teeth not bad; cervical glands palpable, as were the inguinal and subaxillary glands. *Chest* well developed, expansion good and equal on both sides. Diminished resonance over both apices, most marked on right side. No change in tactile or vocal fremitus. Roughened breath

sounds were heard over both bronchi, soft crepitant râles were heard over both apices, more marked on right side anteriorly. *Heart:* Slight accentuation of second pulmonary sound, otherwise negative. *Abdomen:* Liver normal. Speen slightly enlarged and tender.

Laboratory Examination showed Wassermann negative, red blood count normal, hemoglobin 90 per cent, white blood count slight increase to 8,000, differential smear showed an increase in mononuclear cells and negative for malaria or other parasites.

Sputum, stained with carbolfuchsin, methylene blue, was negative for tubercle bacilli but showed the presence of large numbers of large spiral organisms stained slightly pink. Another stain, made by simply staining with carbolfuchsin and washing in water, showed the organisms very well. They were by far the predominating organisms present. No fusiform bacilli were present, the other organisms found being staphylococci and streptococci and a short gram-negative bacillus, probably Pfeiffer.

Course of the Infection.—The patient was given an expectorant cough mixture with an immediate aggravation of the symptoms. The fever continued for about six days showing slight remissions, but, after patient was given Fowler's solution with hypophosphites, the cough and sputum diminished and fever gradually fell to normal. After about ten days' treatment no spirochetes were found in the sputum.

Routine examinations were now made on all natives entering the hospital suffering from bronchitis and soon a second almost identical case was encountered.

CASE 2.—A male, Colombian, apparently forty years of age.

History of the Infection.—The patient had been subject to similar attacks, generally following exposure and always starting with symptoms of a cold. Chills and fever were always present, with the expectoration of considerable foul smelling mucoid sputum, and no hemoptysis.

Examination.—The *neck* showed enlargement of the cervical lymph glands. The *chest* revealed some dullness over the right upper lobe anteriorly with moist râles over the same area. Heart sounds were normal. The *abdomen* showed no abnormal findings.

Laboratory Findings.—Blood, red count, 4,500,000; white count, 9,500; hemoglobin 80 per cent. Differential count showed an increase in the large mononuclear lymphocytes. No abnormal changes in the red cells, no malarial parasites were found. *Sputum* showed numerous spiral organisms. Stained by steaming with carbolfuchsin; no tubercle bacilli were present. A few short bacilli were found. Dark-field examination showed these spirochetes to be actively motile, resembling the mouth spirilli.

Treatment.—The patient was put on Fowler's solution and hypophosphites; the symptoms rapidly abated and returned to normal in one week.

The following cases were observed by the writer in Toledo, Ohio.

CASE 1.—Mr. B. C., ex-soldier, age twenty-seven years.

Family History.—Father alive and well. Mother had been sick for three years, had pneumonia with delayed resolution, abscess of the lung and empyema. Two brothers alive and well. No tuberculosis in the family.

Past History.—Has had ordinary diseases of childhood, including diphtheria. No other serious illnesses; has occasional attacks of cold and tonsillitis.

Present Complaint.—Pain in the chest, cough, expectoration of large quantities of blood. Had been well up until a few months before leaving the service, when he had a similar attack with pain in the chest, cough and hemorrhage. Was confined in a hospital in camp, recovered rapidly. At this time a diagnosis was made of pulmonary tuberculosis, although at no time were any tubercle bacilli found in the sputum. After dismissal from the service he had another attack with hemorrhage as the main symptom. I first saw this patient May 1, 1919; he was expectorating considerable blood, spitting up probably a pint altogether. With proper treatment bleeding stopped and examination was made the next day.

Examination showed a well nourished and well developed male; the head was generally negative. The throat and tonsils were inflamed. The teeth in good condition; the cervical glands were palpable. Chest well developed, expansion normal, no sinking in of the supra-

clavicular spaces. Percussion right lung normal, left lung showed an area of dullness over the upper lobe below the clavicle, some dullness being noted posteriorly over base. Some râles were heard over these areas. No other important pulmonary findings. Heart was negative except for slight accentuation of second pulmonary sound. Abdomen and extremities were normal. The pulse was 80, respiration 22, temperature 99 degrees.

Laboratory Findings.—Throat cultures were negative except for ordinary organisms. Urine normal. Sputum examination showed blood cells, pus cells, mucous shreds and bacteria. Fontana stained smears showed numerous spiral organisms. No tubercle bacilli or elastic tissue was found.

Treatment.—Patient was confined to bed; was given Fowler's solution and hypophosphites with iron and quinine. He improved rapidly under this treatment, and after one month the lung findings had cleared almost entirely. No spirochetes were found in the sputum.

Subsequent History.—I was able to communicate with this patient for several years, all of which time he has remained well and has had no more attacks.

CASE 2.—Mr. J. W., age nineteen years.

Family History.—Nothing of importance in the family history; no tuberculosis.

Past History.—Ordinary diseases of childhood; no other serious illness.

Present Illness.—Started two months ago with sore throat, huskiness of the voice, cough, developed chills and fever, with headache, backache and gastric disturbances. Ran a fever for ten days, became better but cough and huskiness of the voice persisted. Has spit up quite a bit of dark brown material.

Examination showed head generally negative; teeth were in poor condition, throat inflamed, and the cervical glands prominent. Chest showed diminished resonance and moist râles over both apices, the right apex being more involved. Heart sounds were normal, abdomen and extremities were negative. Temperature 99.6 degrees, pulse 84, respiration 22.

Laboratory Findings.—Throat cultures were negative except for ordinary organisms. Urine examination was negative. *Sputum* examination negative for tubercle bacilli, positive for staphylococci, diplococci and streptococci; a few bacilli and numerous spirochetes were found.

Treatment.—Patient given arsenical treatment, later iron and quinine, with rapid improvement and apparent complete recovery. No subsequent attacks occurred.

CASE 3.—Mr. S. A. S., age fifty-eight years.

Family History.—Nothing of importance in the family history.

Past History.—Had ordinary diseases of childhood and pleural pneumonia on right side. Had an attack of influenza several years ago from which he never fully recovered. Has had a chronic nasal infection. Has been suffering from attacks of influenza for several years, being confined to bed a week or more at a time. Has had herpes zoster of more or less chronic nature. Several months previous to this examination patient had an attack of pain in the chest with fever and cough, with increased expectoration lasting about ten days. Recovered somewhat, but cough persisted and he still expectorates considerable slightly blood-tinged sputum. No night sweats have occurred at any time.

Examination showed a well developed adult male, head negative except for a few blotches of herpes on the left cheek. The teeth were in fair condition, the throat and pharynx reddened. No sinus involvement was found. Chest was fairly well developed, resonance diminished somewhat over the right middle and upper lobes, no râles were heard. The right apex somewhat dull and areas of crepitation were heard. Heart, a soft systolic bruit was heard over the aortic area and the heart seemed slightly enlarged. Abdomen and extremities were negative. Pulse 90, temperature 100 degrees, and respiration 20.

Laboratory Findings.—Urine examination was negative. Red blood count 4,800,000; white blood count 8,500; hemoglobin 85 per cent. Sputum examination showed pus cells and numerous bacteria, including staphylococci and streptococci, tetrads and Spirocheta bronchialis. Cultures were attempted to try to cultivate this spirochete, but the efforts were not successful.

Treatment.—Arsenical medications with iron, quinine and hypophosphites caused a clearing of the sputum and some improvement. We lost track of this patient, and the ultimate outcome is not known.

CASE 4.—Mr. F. W., age twenty-eight years, ex-soldier. Original diagnosis, pulmonary tuberculosis.

Family History.—Nothing of importance; negative for tuberculosis.

Past History.—Always well as a child and boy, except for ordinary childhood diseases and frequent attacks of tonsillitis. Tonsils were removed in 1915.

Present Complaint.—Nervousness, headaches, loss of weight, cough and numerous hemorrhages from the lungs. Has had attacks of fever and has frequent night sweats. These symptoms are of about one year's duration.

Examination.—Patient shows some evidence of loss of weight and is rather pale and anemic in appearance. Head: right ear is discharging and has total deafness on this side; other ear normal. Eyes are normal. Teeth are in bad condition. The throat is red and inflamed. The cervical glands are easily palpable on both sides. The chest shows poor expansion, especially on the right side. The supraclavicular spaces are sunken and the intercostal spaces are depressed. There is a dullness over the right lung with bronchial breathing and crepitant râles. The left lung shows scattered areas of dullness with crepitant râles. The heart is slightly enlarged; no murmurs. The abdomen and extremities are negative. Pulse 70, temperature 99 degrees, respiration 20.

Laboratory Findings.—Many examinations of the sputum were made. Pus, epithelial cells, mucous shreds and numerous bacteria, including all the forms of cocci, together with numerous bacilli and spirilli. At no time were tubercle bacilli found. The spirilli were only faintly observed with ordinary staining methods, but the Fontana method showed them to be present in large numbers in every field. The urine was normal.

Treatment.—The patient was not treated by the writer, and to my knowledge no arsenical medication was given. This case remained in the hospital for about one week; was not confined to bed; had some cough, no hemoptysis. This case was reported to the physician in charge as probably pulmonary tuberculosis with a secondary spirochetosis.

CASE 5.—Mr. C. S., age twenty-seven years, ex-soldier. Original diagnosis, pulmonary tuberculosis.

Family History.—Negative except that the grandfather on mother's side probably died from tuberculosis.

Past History.—Has had the ordinary diseases of childhood. Had typhoid fever and suffered from asthma while a boy. No serious illnesses since then until three months after discharge from the army. Smokes many cigarettes daily. Venereal history negative.

Present Complaint.—General weakness, cough and expectoration of a greenish sputum, loss of weight, some fever in the afternoon. Has all the classical symptoms of tuberculosis. Duration about two years; has occasional night sweats. Has not noticed any blood in the sputum.

Examination showed average development, fair nutrition, slight anemia. Head is negative, except that the teeth are in poor condition and the tonsils and pharynx are inflamed. The cervical glands are palpable on both sides of the neck. The chest is fairly well developed with some depression of the supraclavicular spaces, expansion fairly well equal on both sides, breathing regular, not hurried or dyspneic. Breath sounds are clear anteriorly with some few râles heard posteriorly over both bases, especially at the end of deep inspiration. Heart normal. Genitalia negative. Extremities negative. Pulse 72, temperature 99 degrees, respiration 20.

Laboratory Findings.—Daily sputum examinations were all negative for tubercle bacilli, but contained staphylococci, streptococci and diplococci. Many bacilli and numerous spirilli were found. These spirilli were of the Castellani type and stained only with the silver nitrate method. No fusiform bacilli were noted in the sputum. Red blood count 3,286,000; white count 10,860; hemoglobin 65 per cent. Differential count: polys 65 per cent, large lymphocytes 20 per cent, small lymphocytes 12 per cent, transitionals 3 per cent.

Subsequent History.—The temperature while in the hospital was subnormal, except for

one elevation to 100.8 degrees, after which it rapidly returned to subnormal. The pulse ranged from 60 to 90 per minute. Respiration ran around 20. The patient was not confined to bed and was discharged after three days. The diagnosis of bronchial spirochetosis was submitted to the physician in charge.

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DISCUSSION

Dr. F. H. Lamb.—I would just like to ask two questions about spirochetosis. In this condition has Dr. Ramsey observed an increase in size and an apparent increase in the ability of the organisms to take up stain in cases of acute Vincent's angina and why do acute attacks of Vincent's angina occasionally develop in cases of chronic syphilis during the course of administration of salvarsan? It is held by some that salvarsan and neosalvarsan are specific remedies for Vincent's angina.

Dr. A. H. Schade.—I had two cases which the clinicians had diagnosed tuberculosis. I could not find tubercle bacilli in the smear. We were able to demonstrate definitely the spirochetes. They both made rapid recovery and are perfectly healthy at this time, after three years. One of them had watery, bloody sputum; the other's was just blood streaked. They had a typical picture of tuberculosis. One case was treated with Fowler's solution and the other with neoarsphenamine. There was no choice between the two remedies in these two cases.

Dr. H. J. Nichols.—The subject deserves consideration by the Society because there seems to be a very definite entity which, as Dr. Ramsey has said, is due to spirochetes. There may, of course, be some underlying disease which predisposes to spirochetosis in the bronchi. During the epidemic of influenza there was definite evidence that in some cases of secondary pneumonia there were a great many spirochetes in the lungs. I think this condition should be watched and apparently it can be terminated promptly with the arsphenamine.

The cases we have seen have been characterized by serous and bloody expectoration. This is a very timely paper.

Dr. O. Lowy.—I would like to ask Dr. Ramsey whether or not he used gentian-violet in staining. I have used it in all my stains, and I have found a great deal of Vincent's angina.

Dr. Thomas L. Ramsey (closing).—Regarding the staining characteristics of the organism: the spirillum of Vincent's angina stains fairly well with all ordinary aniline dyes and can be seen readily in smears stained by Loeffler's methylene blue. The Spirocheta bronchialis can only be stained, so far as is known, by silver nitrate methods and by steaming carbolfuchsin. I am of the opinion that bronchial spirochetosis is a distinct entity. In regard to attacks or exacerbations of attacks of Vincent's angina brought on by administration of the salvarsan, I have never seen this occur and cannot see any possible connection with salvarsan administration and Vincent's angina. Exacerbations of inflammation of the throat may occur in syphilitic throats immediately following salvarsan administration; this is then followed by almost immediate improvement of the condition.

PNEUMOCOCCUS ANTIBODY SOLUTION*

By F. M. Huntoon, M.D., Glenolden, Pa.

SEPARATION of the immune body from the other serum constituents has been an ideal since the discovery that so-called immune serums contained a substance which, when brought into contact with the homologous antigens, caused certain effects which we have called antibody action.

Such separation was desirable not only for practical reasons, therapeutic usage, but also for the possibility that it might lead to an identification of this antibody substance, which shows in its specific affinities one of the most remarkable phenomenons of nature.

A serum may be regarded as being a colloidal solution containing water, salts, and certain protein substances, which are designated as euglobulins, pseudoglobulins and albumins. An immune serum is a similar mixture plus the antibody substance. In fact we have some evidence that antibody really acts as a foreign body and is eliminated as such.

If, to an immune serum, a ponderable homologous antigen (bacteria) is added, a combination promptly takes place between the antigen and the antibody; the so-called sensitization process or the absorption phenomenon. If the sensitized antigen is removed from the serum by centrifugation or otherwise, and the traces of serum which remain are removed by washing with salt solution, we have remaining a combination of bacteria and antibody free from all gross evidences of the original protein environment of the antibody.

In other words, the antibody has been removed from the other serum constituents. If means could be found to separate the attached antibody from its combination with the antigen, an antibody solution could be obtained free

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20 to 23, 1925.

From Mulford Biological Laboratories.

from the protein material which accompanies antibody in immune serum. Such a procedure has been found and utilized in the preparation of the pneumococcus antibody solution. Briefly the procedure is as follows:

To a polyvalent pneumococcus serum containing antibody against types I, II, and III is added pneumococci of the three types; this is allowed to sensitize in the ice box. The organisms are then removed by centrifugation and are washed twice with ice cold salt solution. They are then emulsified in salt solution and an amount of alkali added in proportion to the number of organisms present. After allowing it to stand, usually, overnight, the material is again centrifuged and the supernatant fluid is removed and saved. On testing this fluid it is found to contain both agglutinins and the protective pneumococcus antibody. The solutions at this stage are somewhat opalescent, because of dissolved bacterial proteins. By various procedures, including storages at low temperature and careful changes in the P_H, most of this material can be removed without materially affecting the antibody contents. The solutions are finally cleared and rendered sterile by passing through Berkfeld filters.

We now have a clear, water colored solution which exhibits all the antibody action of the original serum. That it contains no large amount of serum, however, is shown by the attempt to sensitize guinea pigs with such solutions. When subsequently tested by the intravenous injection of horse serum, approximately 50 per cent of such animals fail to show any evidence of anaphylaxis. This indicates that any serum protein present must exist in very small amounts. Further, in the treatment of over 800 cases of pneumonia with this material we have not in a single instance seen evidences of serum sickness or anaphylaxis, although it has been given to those known to be sensitive to horse serum.

We have in our experience prepared solutions which failed to give any of the known chemical tests for proteins, but still retained antibody action. We have determined, moreover, that antibody under these conditions resists certain deleterious influences to a greater extent than the antibody in the serum. For instance, the influence of incubator temperature reduces the antibody contents little or none over a period of a month, in fact even the addition of trypsin brings no apparent change. Either antibody is not a protein substance susceptible to trypsin or the action of the trypsin is blocked by some other constituents of the solution. Such antibody solutions are not entirely precipitable by ammonium sulphate even when high as 90 per cent saturation is employed.

Antibody, when attached to antigen, is not destroyed by long immersion in absolute alcohol. On the other hand the antibody in such solution is susceptible to the influence of preservative, such as phenol and tricresol. We can say that the tests available tend to show antibody to be a nonprotein substance, but this is far from being proved.

In the original therapeutic work which was undertaken with these solutions, the administration was by the intravenous route and this administration was almost invariably accompanied by a reaction simulating the so-called foreign protein shock (chill, fever and sweating). Such reactions, while

occasionally alarming, were seldom dangerous. In hospital practice, they were not regarded too seriously. The question arose at this time as to the causation of the reaction and its influences on the therapeutic results obtained. There is no doubt that, combined, with the antibody action, it results in many dramatic cures. Following the observation that antibody injection under the skin of rabbits rapidly appeared in the circulation, a certain number of early cases of pneumonia were treated by the subcutaneous route. The results not being as favorable as was hoped, although the reaction was absent, led to further experimental investigation and brought about the discovery that the antibody in such solutions when placed under the skin of the human subject did not appear in the blood stream in any large quantity. This is not true in all cases, particularly in children. It caused, however, the abandoning of the subcutaneous route.

Investigations were undertaken to discover if it was possible to so prepare this solution that the intravenous injection would not be followed by a reaction. The solution of this problem apparently consists in using the greatest care to avoid any extraneous bacterial contamination at any stage of the process. The entire procedure is now conducted at ice box temperature and the majority of the antibody solutions so prepared have been free from the shock factor. The clinical trial of such shockless antibody solution has shown the same beneficial effect in early cases that were noted with the earlier solutions but without the dramatic features of the sharp rising temperature with the immediate fall to or about normal. The temperature falls gradually over periods of two to three days with a coincident improvement in all the symptoms. A very recent development has been the discovery of a rather simple method by which such solutions may be concentrated. It has been found possible to produce a concentration of as much as forty times. While this development is still very much in the experimental state, it is hoped that instead of administering the rather formidable dose of 50 to 100 c.c. a similar or larger dose of antibody may be given in a 5 to 10 c.c. dose.

Six years of experience indicates that the preparation of such solutions is the route by which the ultimate nature of antibody will be determined and that it offers at present the best method for the therapeutic use of specific immune body.

DISCUSSION

Dr. John A. Kolmer.—This interesting summary of Dr. Huntoon's work is now open for discussion, and I may state that the method developed constitutes a useful start in the perfection of a technic for the dissociation of antibody in a pure state for the highly desirable studies on the chemistry of antibodies. During the past year Dr. Uchida in my laboratory has been interested in developing the method for the isolation of complement-fixation antibody for chemical studies.

Dr. Robert A. Kilduffe.—I should like to ask what is the dosage of this preparation. I was also much interested in the medium mentioned for the cultivation of the pneumococcus, and I should like to know whether its preparation and formula have been published.

Dr. A. C. Abbott.—I am more or less familiar with Dr. Huntoon's work. Manifestly, it is still in the experimental stage, and by the complexity of its nature it originates questions that must be more convincingly answered. For instance, one such question,

at least, relates to the interpretation of the action of the alkali used. It is difficult for me to believe that that action is limited to disengaging the antibody from the antigen, the bacteria. I cannot avoid an opinion that in addition to that function there must be a direct action upon either the bacteria themselves or the antibody, or both. That question, however, if of importance, can safely be left to Dr. Huntoon for answer.

Dr. David H. Bergey.—Dr. Huntoon's work is of great interest, because fundamentally it serves to extend the clinician's methods of treatment. The desirability of placing in their hands an antibody solution is so great that it is not surprising that many clinicians have continued to use this solution in spite of undesirable reactions having accompanied an earlier preparation. The elimination of this has been a large part of the object in perfecting his solution.

Dr. Robert A. Keilty.—We have been using this from the beginning. Our results have justified the continuance of it. It is rather expensive. What are the future possibilities of a still further reduction in price?

Dr. F. M. Huntoon (closing).—The first question I believe was about a precipitate forming in the solution. Certain of these solutions will after a time form a precipitate which goes into solution again when heated to body temperature.

The question of medium I can answer very quickly. This formula has been published in the Journal of the Society of Experimental Biology.

The question of dosage was originally based on the amount of antipneumococcus serum that had been used. During the first two years, Dr. Cecil did four hundred cases; he used large doses. Dr. Conner at the New York Hospital gave comparatively smaller doses, 25 c.c. and 50 c.c., the second dose of 50 c.c. to men. Dr. Conner's results were better from a statistical viewpoint than the others. Men this year like to use 100 c.c. doses and they are generally perfectly safe. If no results are obtained with three or four doses, you might as well stop.

The question of expense: that depends entirely on volume; the cost to the consumer will automatically drop as the production rises. Experimentation on this scale costs a great deal.

Now the question as to whether these solutions are pure antibody or not. No, far from it. We have produced highly potent material which gave none of the chemical tests for proteins, but that material still contains somewhere around 3 mg. of nitrogen per hundred cubic centimeters. The mere statement that it gives none of the chemical tests for protein does not mean that the proteins are not there. My personal opinion is that the antibody is still combined with antigen, that when you take off the antibody a small portion of the antigen splits off with it. It is impossible to prepare it in the pure substance. The fact that it is combined with antigen does not impair its reaction in the least. I doubt very much that there is any antibody in the solution that is in a free state. The question of obtaining absolutely pure antibody, I doubt if it is possible.

THE PROBABLE RÔLE OF THE BACTERIOPHAGE IN STREPTOCOCCUS INFECTIONS*

By L. O. Dutton, M.S., Memphis, Tenn.

THE literature concerning the bacteriophage has few references to the phenomenon of transmissible lysis of the streptococci. D'Herelle[1] mentions that streptococci have been lysed, and McKinley[2] reports that he has adapted a phage that was secured from d'Herelle to the streptococcus. McKinley[3] also reports the successful treatment of a streptococcus lung abscess with the bacteriophage. Aside from these meager details there is little that is enlightening concerning the bacteriophage as a factor in streptococcus infections. The literature also leaves one with the impression that the bacteriophage is not readily isolated from the blood stream and, if it is found there, it remains for a very short period only. The data detailed here is offered because it suggests an interesting phase in the understanding of streptococcus infections, as well as speculations of interest as to the nature and distribution of bacteriophage strains active for these organisms.

The isolation of a bacteriophage active for streptococci reported here came about rather as an accident. A blood culture was taken from a patient who had been operated upon two days previously for a mastoid infection. On this day the temperature of the patient rose to 105° F. Ten c.c. of blood were delivered into a flask containing 200 c.c. of veal infusion dextrose broth, P_H 7.4, and 1 c.c. of blood was delivered into each of five tubes containing 10 c.c. of dextrose agar, P_H 7.2 mixed, and plated. The following morning, fifteen hours after culturing, the flask and plates were examined. There was a cloud in the flask but there were no colonies on the plates. Smears from the flask revealed gram-positive cocci in pairs and in short chains. Transplants were made from the flask to blood agar and to dextrose agar. The cultures were then incubated for another twenty-four hours. At the end of this incubation period, there were still no colonies on the agar plates, nor was there growth on the plants from the flask. A second smear from the flask at this time failed to reveal any bacterial cells at all in spite of long search. After another twenty-four hours growth, the transplants from the flask on blood agar had developed a few very peculiar colonies. They were large, mucoid, and transparent, with a greenish color. Smears of them revealed a streptococcus-like organism with a great many atypical cells. Smears from the original flask at this time failed to show organisms.

An hypothesis to explain the unusual findings was sought by assuming the presence of a bacteriophageous lytic principle in the blood of the patient or carried in the strain of streptococcus itself. Subsequent study of the culture seemed to substantiate the latter assumption. The behavior of the patient was

*From the laboratories, The Methodist Hospital, Memphis, Tenn.
Received for publication, July 9, 1925.

interesting in the light of such cultural findings. After a short period, twenty-four hours, of high temperature, the general condition of the patient became improved, the temperature and pulse rate fell to normal, and in the course of four days, the patient was discharged from the hospital.

A report of the detailed study of the culture will appear in the near future. It is sufficient to say here that, so far as the particular strain of streptococcus involved in this study, transmissible lysis was never obtained. By washing the entire growth from an agar slant into a flask of plain infusion broth, however, a definite and heavy growth took place in eighteen hours that became completely lysed in twenty-four to thirty hours. This phenomenon could be obtained as long as growth was maintained on agar. The bacteriophage became so lytic in its activity, however, that, after the course of two months transplanting, the cultures failed to grow. This phage is to be designated as phage "H" hereafter.

In the absence of transmissible lysis, and in view of our incomplete knowledge of streptococcus cultures mixed with a bacteriophage, it was impossible to conclude that the bacteriophage was the factor responsible for the lysis of the culture. We have since been able to produce experimental transmissible lysis of other strains of streptococci, however, and conclude that we are dealing with a bacteriophage active for these organisms.

Assuming the correctness of d'Herelle's hypothesis as to the rôle of the bacteriophage in recovery from disease, it becomes logical to conclude that the bacteriophage was the factor responsible for the recovery of this patient. The therapeutic use of this phage then becomes the next obvious step.

A patient having a precipitous delivery developed thirty hours after delivery a temperature of 105° F. A blood culture taken several hours later showed, at the end of eighteen hours' incubation, a heavy growth of a streptococcus of the hemolytic type. The general condition of the patient at this time was very grave. One c.c. of phage "H" was injected intravenously. Following the injection there was a rise in temperature of .6° F. There was no general reaction, however. After two hours the temperature began to recede gradually and the patient felt better. The lowest point of the temperature curve for the day, however, was 102.4° F. The following morning, a second 1 c.c. of phage was given intravenously. This was just twenty-four hours after the initial injection. Four hours later a second blood culture was taken and, with the same needle, a third 1 c.c. of phage was given. The patient's temperature persisted at a high level, however, and twelve hours after the third dose of phage 25 c.c. of 1 per cent gentian-violet was given. For the next twenty-four hours the temperature curve was constantly at a high level, and a second dose of gentian-violet was given. Twelve hours after this last dose and fifty-two hours after the initial phage dose, the temperature was normal, and remained so except for one high peak on the third day following. Otherwise the patient went to a rapid and complete recovery. The second blood culture, taken before gentian-violet was given, was sterile after five days incubation.

An analysis of this case leaves us somewhat in doubt as to the relative parts played in the recovery by the phage and gentian-violet. Experiments

were done, however, that are interesting in the explanation of this point. To begin with the organisms isolated from the first blood culture were subjected to the action of phage "H" in a series of passages. It was found that at the eighth passage the tube containing the phage was completely lysed, while the control tube contained a heavy growth. This established the fact that the organism was sensitive to the action of phage "H." The next experiment was to employ a filtrate of the first and second blood cultures as a starting point for a possible phage and subject the organisms to a series of passages. In this experiment, the passages of the second culture showed lysis on the fourth passage, while the passages of the first culture failed to show lysis even after 15 passages. This indicates that there was no lytic principle in the patient's blood at the onset of the disease and that there was such a principle present after innoculation of a bacteriophage. Controls of this experiment were a series of passages from a sterile blood culture of another patient and a series of passages without any initial inoculation of a phagic or probable phagic substance. That is, a normal culture was filtered and passed into a second culture. These controls established that the serum of the blood had no part in the lysing of the organisms and that the organism was carrying no phage mixed with it. This chain of evidence is nearly conclusive that the lytic principle was the factor responsible in a large measure for the recovery of the patient. These experiments are tabulated under Exper. 1.

A second case was chosen to try the therapeutic value of the phage. This was a hemolytic streptococcus infection of about four weeks' duration. The blood culture was positive and the patient had many abscesses in various parts of the body. Those that were visible were in the muscle septa. The patient was given 1 c.c. of phage "H" intravenously. On the next day the patient was not improved. A second blood culture was taken. This culture proved to be positive and the patient died on the fourth day following the phage injection with the symptoms of an embolism.

The experiments done on the previous cultures were repeated with interesting results.

In this series of tests we were not able to obtain lysis of the organisms under any conditions. The passage of the phage that had been given the patient always remained cloudy as did the passage of the blood culture filtrates. Thus it was demonstrated that the organism responsible for the condition of the second patient was resistant to the phage, and that no lytic agent was in the patient's blood. These are detailed under Experiment II.

Without giving the details of other cases it is sufficient to say that the phage has been used intravenously on three other definitely proved streptococcus blood stream infections. Of these three, two patients recovered, one following an acute arthritis with hemolytic streptococci in the blood stream, and one following a septic hemolytic streptococcus infection after a mastoid primary infection. The third patient died after having received the phage intravenously during the end stage of a streptococcus viridans endocarditis. The injections were given as in the successful case detailed above and the experimental results obtained were identical with those given in detail.

The technic of the experiments was as follows: Veal infusion broth ad-

EXPERIMENT I

SERIES	STRAIN OF STREPTOCOCCUS	FILTRATE	PASSAGES 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	428	H	C	C	C	C	C	C	C	L	L	L	L	L	L	L	L	L	L	L	L
2	428	First Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3	428	Second Blood Culture	C	C	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
4	428	Auto Filtrate	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5	428	Sterile Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
6	428	None	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Strain 428 isolated from patient who recovered following injection of phage.
Filtrate H., lysed culture of strain isolated from patient who recovered spontaneously.
Auto filtrate, filtrate of a normal broth culture of strain 428.
"C" indicates normal cloud after 24 hours' incubation.
"L" indicates clear tube after 24 hours' incubation.

EXPERIMENT II

SERIES	ORGANISM	FILTRATE	PASSAGES 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	440	H	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2	440	First Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3	440	Second Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
4	440	Auto Filtrate	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5	440	None	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

H Filtrate, lysed culture of organism 428 after addition of H phage.
"C" indicates normal clouds after twenty-four hours' incubation.

justed to P_H 8 was tubed in 10 c.c. quantities, and, after isolation of an organism, a few transplants through this medium resulted in characteristic streptococcus growth. Most of the strains studied in this medium have given consistent and abundant growth from the very start. It is important that carbohydrates are not used in the medium, as the organisms ferment the sugar to result in a shift of the P_H to the acid side, and it is necessary that the medium remain alkaline to obtain phagic activity. Various methods of utilizing sugars as growth stimulators with calcium carbonate or buffers to control the P_H were discarded in favor of the more constant veal infusion plain broth. When the organisms showed a good growth in this medium a tube was inoculated, and three hours later a second tube was inoculated from the first. This second tube was then incubated for a three-hour period, and a known or suspected phage was added to it. In general 1 c.c. quantities of the phage were used. These tubes were incubated at 37° C. for twenty-four hours, with observation being made at various intervals. There was generally a cloud in all the tubes at the end of nine to twelve hours after inoculation. In the tubes that exhibited lysis of the organisms there remained only a slight sediment at the end of twenty-four hours, while in the control tubes and the tubes showing no lysis there was a marked cloud at this period. At the end of this twenty-four-hour incubation period the cultures were filtered through a Mandler filter. The No. 5 size was found convenient for the small quantities of media used. One c.c. of the filtrite was added to a culture planted as described above from a three-hour culture. This procedure was repeated for the successive passages.

The necessity of inoculating tubes from three-hour broth cultures, and of adding the filtrates to three-hour cultures was recognized after experiments concerning these points. It was found that lysis was not obtained when the filtrates were added to cultures that were older than three hours, and it was found that the three-hour age was best from which to make the inoculations. It has not yet been determined whether the essential factor in this step is the age of the cells that were inoculated or the decreased number of cells that would result from inoculations of such young cultures.

SUMMARY

In surveying these three cases we find that in the initial one a streptococcus blood stream infection occurs that recovers spontaneously without treatment. On study of the organism isolated from this case a lytic principle was obtained that would lyse strains of streptococci. In the second case a blood stream streptococcus infection occurred that was promptly overcome when the phage that was secured from case No. 1 was injected. This was followed by a recovery of the patient, and also, experimentally, a lytic principle was found to be absent from the initial positive blood culture, while from the second sterile culture a phagic substance was obtained that produced lysis of the streptococci isolated from the patient. Case No. 3 was found to have a streptococcus blood stream infection. After administration of the bacteriophage the blood stream still contained the organisms and the patient died. Experiments with the cultures obtained in this case failed to reveal a phagic substance either before or after the administration of the phage. Nor was it possible to obtain lysis of

the strain of streptococcus that was isolated from this case with the phage that was used. In other words the organism present in the first two cases was susceptible to the bacteriophage and the patient recovered, while the organism in the last case was not susceptible to the phage and the patient died.

CONCLUSION

These facts are all in accord with the hypothesis d'Herelle has advanced concerning the rôle of the bacteriophage in infection and immunity.

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LABORATORY METHODS

A TIME- AND LABOR-SAVING PHOTOGRAPHIC APPARATUS*

By Joseph B. Homan, Cincinnati, Ohio

FIG. 1. Apparatus used in making lantern slide, negatives from books, photographs, and charts. Photographs all kinds of medical specimens, instruments, charts and any other objects conveniently laid out flat. With three lenses, 6, 10 and 14 inch focus, enlargements can be made or a 36 inch chart can be reduced to 8×10 inches or smaller. Apparatus is very speedy, taking from twenty to twenty-five pictures from books in one hour.

Description.—Made mostly of 1 inch 1. D. pipe.

Vibration is overcome by braces. Eight 1 inch flanges in wall and ceiling and 6 lag expansion bolts in floor.

Two stands and a pipe ladder to each stand.

When the higher stand is used the lower stand can be swung out of the way, thus enabling the complete board to be used.

Thirteen feet high and takes up less than eight feet square of floor space.

No. 1.—The board, 5 feet by 36 inches, grooved to take light angles. This board is mobile, moving either east or west, north or south, kept parallel with the camera by a device under the board. These motions are obtained by the use of levers, No. 4 and No. 5, which can be reached from either stand, and *while looking onto the ground glass of the camera.* Lever 4 swings to one side when upper stand is used.

The board is laid off with center and parallel lines.

An object can be centered in five seconds.

No. 2.—Light angles; each contains 5 100-watt bowl frosted incandescent lights, each light having a separate switch.

Each angle can be moved forward or back on steel shoes, which slide in grooves on board.

Each angle can be moved up or down on rods and set at the proper height.

The lights are on a mobile board which can be moved in and out inside the angles. Each angle has three different motions to adjust light to insure exactly the proper angle of light.

No. 3.—Camera, Folmer and Schwing enlarging, reducing and copying camera with a five foot bellows extension.

The camera is held perfectly rigid, by a lever and set screw, from the upper stand.

An adjusting screw permits a motion of eighteen inches up or down. If

*From the Department of Medical Art, College of Medicine, University of Cincinnati.
Received for publication, November, 16, 1925.

this is not enough the set screw is loosened and the complete camera is moved up or down on the pipe slide by the drum No. 6 and a counter weight or, as the lens is usually used in the middle compartment which is movable, there is another means of racking the lens up or down.

For the final focusing we have the micrometer focusing screw. There are four different means of securing a focus.

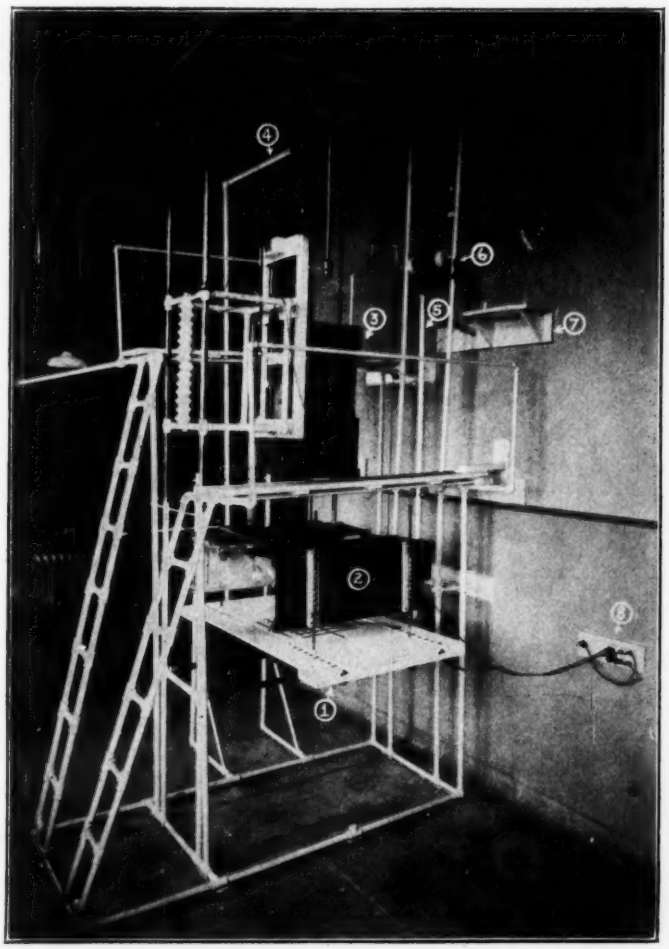

Fig 1.

Nos. 4 and *5.—Levers* which pull the picture to a center on the ground glass in five seconds. They have a point of leverage which makes them very easy to move.

Centering the picture is done while looking onto the ground glass, it being unnecessary to go down the ladder to adjust the book or picture.

No. 6.—Drum used in winding camera up or down.

No. 7.—Shelf upon which unexposed and exposed plates are put.

No. 8.—Switch to control lights.

The apparatus has a wooden rod suspended alongside of camera on which are the various lens distances and exposure time for various plates.

Fig. 2 shows detail of light angles and layout on board.

No. 1.—Side lever.

No. 2.—Light angle showing steel shoes and how lights are focused by aid of pins inside the angles.

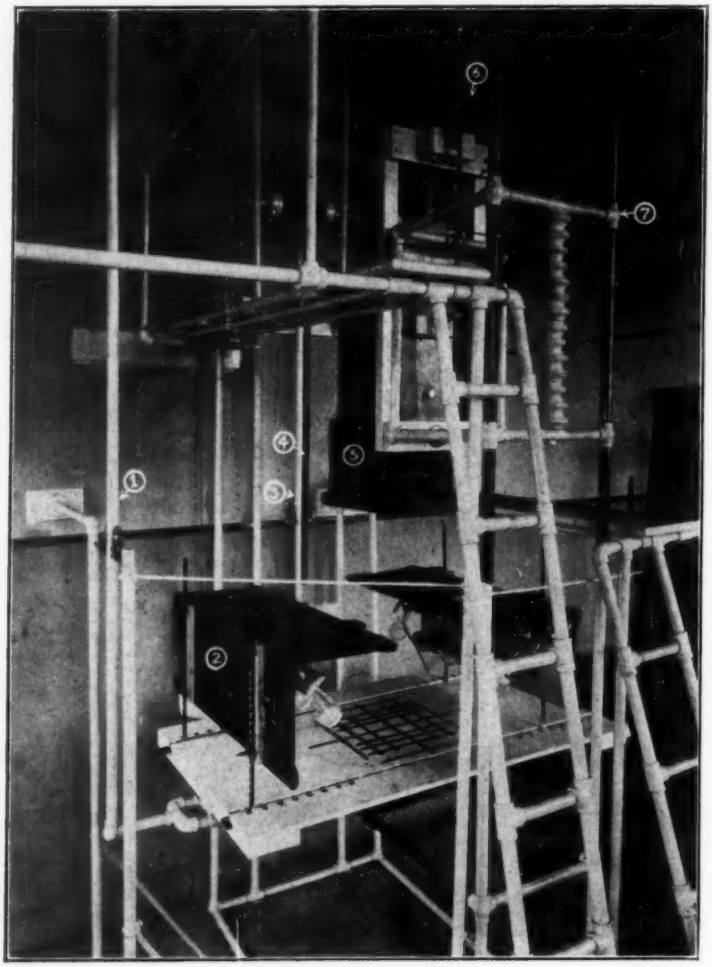

Fig. 2.

No. 3.—Back lever.

No. 4.—Wooden rod with exposure time and size.

No. 5.—Camera.

No. 6.—Adjusting screw for moving camera up and down.

No. 7.—Slide which permits complete camera to move up and down by aid of drum and counter weight.

Fig. 3 shows detail of levers.

No. 1.—Counter weight for camera.

No. 2.—Joints on which the movement of board depends.

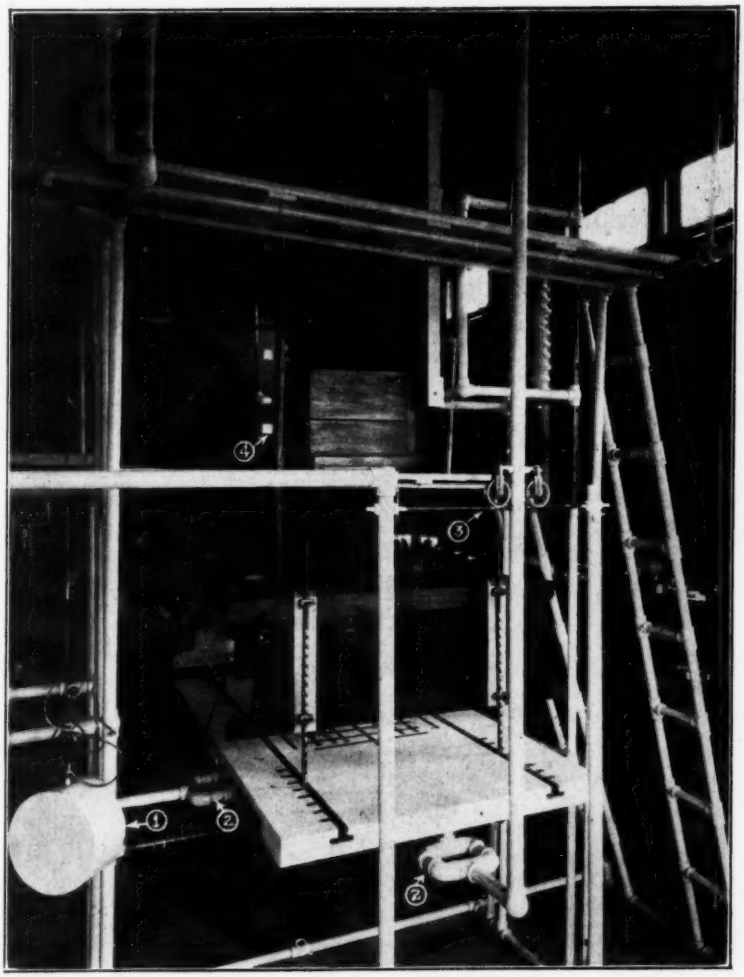

Fig. 3.

No. 3.—Point of leverage, showing pulley which enables lever to ride more easily.

No. 4.—Wooden rod with exposure time and size.

TWO CONVENIENT FORMS OF APPARATUS FOR MICROBLOOD AND MICROURINE ANALYSIS*

By F. C. Koch, Ph.D., Chicago, Ill.

THE two devices described below have been found very convenient and reliable for individual as well as for class use in blood and urine analysis as well as for other purposes. The first is an improved distilling tube for micro-Kjeldahl or microurea distillations, and the second is a convenient device for rapidly and accurately measuring off various reagents.

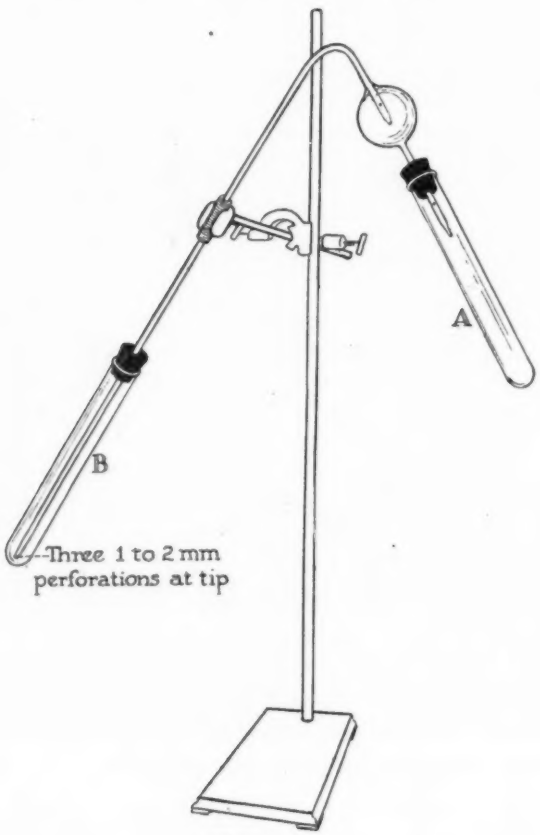

Fig. 1.

Improved distilling tube.—This consists of a regular distilling bulb of the Hopkins type attached to a 19 inch length of ¼ inch tubing at the angle indicated (Fig. 1). The short tube from the bulb projecting into the boiling tube *A* is made as short as possible, allowing space for a ⅛ inch hole in the

*From the Hull Laboratories of Physiological Chemistry of the University of Chicago. Received for publication, October, 1925.

side just below the rubber stopper. This hole ensures smooth boiling. At the very tip of the 19 inch length of ¼ inch tubing are three holes 1 to 2 mm. in diameter. These holes serve the double purpose of ensuring complete absorption of ammonia in the early stages of the distillation and of preventing the too rapid return of the distillate into the boiling tube *A* in case of changes in pressure during the distillation process. The usual micro-Kjeldahl or micro-

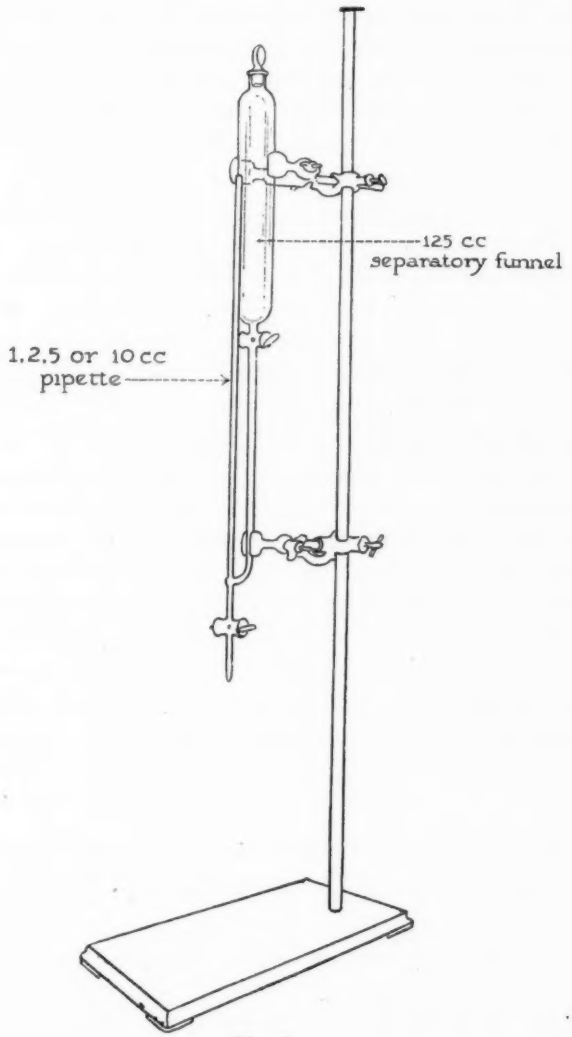

Fig. 2.

urea distillations are easily carried out in three to five minutes to the point where live steam issues through the distillate in *B*.

In the micro-Kjeldahl distillation the 1 c.c. of N/10 H_2SO_4 and 5 c.c. of ammonia-free water are measured into *B;* this is then securely attached to the mounted distilling tube. The latter (well protected by a cushion of heavy rubber tubing) is held in a clamp. To the contents of the digested mixture, consisting mainly of concentrated sulphuric acid, add 7 c.c. of distilled water and

a small knife point of 30 mesh zinc. Cool, and then add by means of a pipette 4 c.c. of the saturated NaOH solution in such a manner that the alkali flows down the side of the tube and forms the lower layer of strongly alkaline solution. Immediately after this addition connect the tube very securely with the distilling tube. Mix the contents of tube *A* thoroughly by shaking at tube *B*, using the clamp holding as a fulcrum. Then at once heat to boiling with a Bunsen burner, allowing the flame to play on the sides of the tube *A* just below the surface of the liquid rather than on the very bottom. Do not boil too vigorously but gently and continuously. When the contents begin to bump and crystallize, remove tube *B*, rinse off the tip of the distilling tube into *B* and then, by applying the flame on *A*, force out the small amount of water which has been drawn in at the tip during the washing. The material is then Nesslerized after appropriate dilution.

The sketch of the other apparatus (Fig. 2) is self-explanatory. Several precautions should be mentioned, however. The graduated pipettes used in assembling this apparatus should be high grade and carefully selected. The graduations on the 1 and 2 c.c. pipettes should be 1/100, and on the 5 and 10 c.c. 1/20 and 1/10 c.c., respectively. The length covered by the graduation should be 200 to 210 mm. for the 1 and 2 c.c. pipettes; 160 to 180 mm. for the 5 c.c. pipette, and 210 or more mm. for the 10 c.c. size. This apparatus we have found very valuable for measuring indicator solutions for P_H estimations and for measuring numerous reagents in microblood analysis. It has been found very satisfactory for measuring the cyanide solutions in uric acid estimations and for the titration of blood calcium by the permanganate method. In such very delicate titrations as the latter it is best to draw out the tip of the burette stopcock to a very fine point. One can easily obtain a tip of so small a bore that it delivers 100 to 110 drops per cubic centimeter. I have recently simplified this apparatus by substituting a three-way stopcock of the oblique bore type for the lower stopcock on the apparatus as illustrated. By so doing the stopcock on the separatory funnel part can be omitted.

A STABLE AND CONVENIENT UREASE REAGENT AND A MODIFIED BLOOD UREA METHOD*

By F. C. Koch, Ph.D., Chicago, Ill.

THE advantages of a stable solution of urease are, of course, obvious to all. In order to overcome the serious objection of instability so often encountered in the use of various convenient urease preparations, the well-known preservative action of glycerol toward many enzymes was applied to urease and found to fulfill the requirements.

The following formula has given in our hands very stable and satisfactory solutions of urease. Shake 15 grams of permutit with 200 c.c. of 2 per cent acetic acid. Decant the aqueous phase and wash two or three times with

*From the Hull Laboratories of Physiological Chemistry, University of Chicago.
Received for publication, October, 1925.

distilled water. To the permutit then add 50 c.c. of N/1000 H_2SO_4 and 30 grams of jack bean meal (Arlington Chemical Co.). Shake gently for one hour, then add 150 c.c. glycerol and mix very thoroughly. Filter through a creased filter. The filtration is slow and the product turbid. It may be necessary to change the filter paper after a few days in order to aid filtration. The filtrate is, however, very active and stable. The equivalent of 0.03 to 0.05 c.c. of such a glycerol extract of jack bean meal has been found to convert 2 mg. of urea nitrogen completely into ammonia in a 5 c.c. volume at 45° to 50° C. in fifteen minutes. Such extracts have been found to retain their full activity for as long as one year although stored at room temperature and exposed to the usual laboratory illumination. For ordinary blood urea estimations 1 c.c. of the 1:10 *freshly* diluted glycerol solution for 5 c.c. blood filtrate has been found reliable.

MODIFIED BLOOD UREA METHOD

The modified blood urea method can be employed only where sufficient blood is available to permit of preparing a separate blood filtrate for this specific purpose. The procedure is as follows:

Into a clean 1 × 8 inch pyrex test tube introduce accurately 2 c.c. of the well mixed blood. To this add 9 c.c. of distilled water, 2 drops of Folin's phosphate solution and 5 c.c. of the 1:10 *freshly* diluted glycerol urease solution. Mix well, stopper the tube with cotton and allow it to incubate for fifteen to thirty minutes at 50° C. Then add 2 c.c. of the 10 per cent sodium tungstate solution and complete the precipitation of the proteins by the usual addition of 2 c.c. of the 2/3 N sulphuric acid. Shake well, filter, and transfer 5 c.c. of the *clear* filtrate to a 1 × 8 inch pyrex test tube. Add the 2 c.c. of saturated borax solution, a small knife point of 30 mesh zinc, and distil with the tube described in the previous article.

This procedure enables one to carry out a smooth distillation without the usual serious foaming. We have found this modification very satisfactory for the routine laboratory classes, whereas by the older procedure we usually obtained very low results because of poor distillation.

AN IMPROVED EXTRACTION TUBE FOR CHOLESTEROL DETERMINATION*

By S. L. Leiboff, Ph.B., New York

IN July, 1925, I described in this Journal a special apparatus for the extraction of cholesterol from blood. It consisted of a small test tube constricted in the middle at the 5 c.c. mark. Above the constricted portion of the tube was placed a filter-paper disc containing the blood. The disc was perforated in the center by a small opening in order to allow the free passage of the chloroform.

*From the Biochemical Department of Lebanon Hospital Laboratory, New York
Received for publication, November 26, 1925.

The tube was then attached to a condenser and extracted with chloroform.

On account of the difficulty in preparing the filter-paper discs, it was found advisable to use straight pieces of filter paper without openings in the center. These are easily prepared from Whatman's double thickness extraction thimbles by cutting them up into 19 millimeter squares. These are just large enough to absorb 0.25 c.c. of blood, which is the required amount for the determination of cholesterol. The four corners of the paper are turned

Fig. 1.—Extraction tube.

upward so that the squares fit in the extraction tubes. About 18 squares of paper are obtained from a 22×80 mm. extraction thimble.

In order to allow the chloroform to pass freely without being obstructed by the filter paper, a side neck, connecting the upper and constricted portions, was added to the tube, as shown in the accompanying figure. The filter paper thus lies between the upper and lower openings of the side neck through which the chloroform passes without obstruction.

These tubes are now used instead of the original type in the standard cholesterol apparatus.

A MICROSYRINGE*

By M. S. Marshall, Ph.D., and Ralph L. Burd, M.E., Lansing, Mich.

ALTHOUGH the screw thread micrometer principle has been applied before now in the accurate control of syringe measurements,[1, 2] the market for such instruments is so limited that commercial houses have not undertaken their construction. Since the worker needing it must make the instrument himself, a description of a microsyringe which incorporates certain features not possessed by those previously described is herewith presented. Its advantages or disadvantages are left to the reader.

DESCRIPTION OF THE INSTRUMENT

The essential parts of the instrument consist of a screw thread micrometer head (Brown and Sharpe), a tuberculin syringe, and an ordinary hypodermic needle. The essential principle is the controlled gradual thrust of the syringe plunger over small and accurately measured distances.

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*From the Michigan Department of Health.

In case anyone wishes such an instrument, but has not the facilities for making it, Mr. Burd will be glad to undertake its construction.

Received for publication, January 6, 1926.

The micrometer head, *M*, is metrically graduated over a distance of 25 mm. (maximum thrust) in divisions calibrated to 0.01 mm., and accurate to 0.0025 mm. by interpolation. The screw thread type of micrometer head has, instead of the usual flat face of the regular type, a pointed face, as appears in the photograph. It is essential that there be perfect contact between the micrometer head and the syringe plunger, which is insured by the accurately centered metal point. The accuracy is greater than that involved in polishing the head of the syringe plunger in a plane perpendicular to the axis of the plunger, or in drawing the syringe plunger to an accurately centered point. This micrometer head is set in a hole in a piece of inch brass rod reamed to fit. In order to insure rigidity, a thumb set screw, *D*, is inserted. On each side of

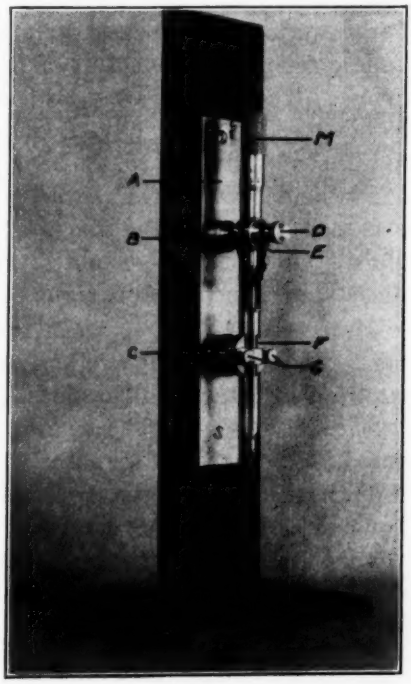

Fig. 1.

this micrometer holder are placed brass screws, *E*, set tight, which serve only to hold rubber bands which are attached to the syringe plunger, keeping firm contact between the plunger and the head. Contact of this sort pushes the plunger directly, with no turning.

The syringe itself is set on a brass rod, *F*, through which a hole to fit has been bored, the rod then being cut off through the center of the hole, leaving a semi-cylindrical groove for the syringe. To clamp the syringe in, a piece of heavy strip brass is bent to fit, and a piece of rubber tubing, cut in semi-cylindrical form is cemented in. This brace is fixed to the main stock by means of two knurled thumb screws, *G*, long enough so that the syringe may be loosened easily without removing the piece.

The vertical position of the micrometer head is insured by the large shoulder of the brass fixture, *B*, pressing against the flat metal strip facing,

A, used on the board. With the exception of the thumb screws, *D* and *E*, this piece, *B*, is made from one section of brass rod. From *B* this piece, 3/8 inch in diameter and threaded, extends through the board, being secured by a large thumb lock nut on the back of the board.

The syringe holder also extends through the board to be secured by a thumb lock nut in the rear, but the solid piece corresponding to the shoulder, *B*, of the micrometer holder is replaced by a similar thumb lock nut. The syringe may, therefore, be locked in a position centered under the point of the micrometer, being so made as to be set out or in from the board by means of the lock nuts, and movable sideways by a rotary motion. By loosening either of the set screws, the free rotary motion enables the swinging of the syringe out from under the micrometer for removal or refilling. Inasmuch as the useful stroke of the syringe plunger is greater than 25 mm., the maximum thrust of the micrometer, a syringe full of liquid may not be used without resetting the syringe. The syringe may be loosened and set higher. Perhaps it is preferable, however, to place a vertical slot instead of a hole through the plate *A* and through the board. The large thumb lock nuts enable one to fix the syringe holder very firmly, whereas the syringe position in the holder is best guaranteed by having the flange on the top of the barrel resting on the metal holder. Obviously the slightest slip of the barrel will make a measurement valueless, and such a slip might easily pass unnoticed.

The syringe may be either an ordinary 0.5 c.c. tuberculin syringe, or a 1.0 c.c. tuberculin syringe. Both fit equally well, since the difference is in the size of the plunger, and not in external dimensions. We have also another instrument built for a 2.0 c.c. Luer syringe, for use when greater amounts of liquid are to be measured. The best needles for the purpose are platinum-iridium needles, squared at the end. We have, however, been successful with ordinary steel needles, squared. A stroke of the syringe plunger of *m* millimeters ejects *n* c.c. of liquid with any one syringe, quite regardless of the size, shape, or condition of the needle, and a steel needle, carefully cared for has given us good results.

ACCURACY OF THE INSTRUMENT

Illustrating the accuracy of measurment of liquid, the figures for calibration of a 1.0 c.c. tuberculin syringe may be given. Glycerin was used for calibration, having a tested density of 1.248 at 21° C. Amounts ejected by turning 500 scale divisions on the micrometer (or 5 mm. in vertical push on the syringe) were weighed. Twelve weighings, covering the length of the syringe twice, gave as the weights in milligrams: 139.3, 138.9, 138.8, 138.8, 138.6, 139.0, 138.9, 138.9, 138.9, 138.8, 138.9, and 138.8. The average weight is 138.88 ± 0.047 mg., or 0.03 per cent error, as the mean average of the mean. The mean error of each determination is 0.164 mg., or 0.12 per cent error. On this basis one small scale division on the micrometer (0.01 mm. push) ejects from this syringe 0.0002226 c.c. This figure is of the nature of a factor; naturally, so small an amount would not be accurate to the number of points to which the figure is carried. Thus, if one wishes to measure 0.021 c.c., the syringe plunger must be pushed 94.3 one hundredths millimeters, or

that number of scale divisions. Five scale divisions eject only slightly over 0.001 c.c. It is thus apparent that the control over small amounts is good. With the 0.5 c.c. tuberculin syringe, our syringe gives for 0.021 c.c. 192.8 scale divisions, or approximately twice as many as needed with the 1.0 c.c. syringe. This smaller syringe is, therefore, capable of handling amounts one-half as great as the larger syringe with virtually the same accuracy.

That the liquid be flush with the tip of the needle at the start of measurement is essential; in our experience touching off squarely with the flat dry surface of a glass slide, or observing the surface with a hand lens, are satisfactory. In the latter method, a droplet formed at the tip of the needle may be drawn back by reversing the micrometer until the liquid is square with the opening by actual observation with a hand lens.

SUMMARY

A microsyringe is described, capable of measuring small amounts of liquid, from less than 0.001 c.c. to 1.0 c.c., with an error of not over 0.2 per cent, and, for appreciable amounts (e.g., 0.02 c.c.), less than 0.1 per cent.

The authors wish to acknowledge the careful work of Miss Esther Shultis in checking the calibration of the syringe, thereby virtually eliminating a personal factor in the calibrations.

REFERENCES

[1]Trevan: Lancet, April 22, 1922, i, 786.
[2]Bayne-Jones: Jour. of Immunol., November, 1924, ix, 481.

SOME USEFUL APPARATUS*

By Max Shaweker, M.D., Dover, Ohio

BEING situated at a considerable distance from a supply house, we are frequently called upon to rig up makeshifts of one sort or another. At times these makeshifts prove to be equal to the standard apparatus.

The first piece is a transfusion apparatus which has proved to be quite positive in action and almost a one man affair. It appears to be just the opposite of the latest types recommended in that it does not hurry the operation or move the blood rapidly. We have tried several of the valves recommended for directing the flow of blood from the donor to the recipient, but they have all proved to be unsatisfactory, either favoring clotting or not being air tight. They seem to require the blood to "squirt" through some valve or other when the natural motion of blood is one of smooth flowing. In our apparatus (Fig. 1) we have reamed out all the metal parts so as to provide a large bore and to favor flowing rather than "squirting." Then our syringe is the simple Bransford-Lewis type of 15 c.c. capacity which is operated by one hand leaving a free hand for adjustments and valving

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, May 20 to 23, 1925.

duty. The tube of this syringe can be paraffined if desired. The fingers used on the tubing, instead of clamps or valves, afford a sure working valve operation. The irrigation solution, either isotonic salt solution or a weak citrate solution, is placed in the upper jar and the conductor tube is connected to the large bore adapter (D) of the donor's needle or cannula, as the case may be. Another conductor tube is placed to the adapter (R) of the recipient's needle. This conductor tube is carried to a height just below the irrigating jar where a Y-tube (Y) is interposed so as to maintain a slight positive pressure in the tube system. These two tubes are kept parallel and are of the same caliber of tubing so as to be easily operated simultaneously with one clamp. Small caps (C) for the needle adapters are made by sealing

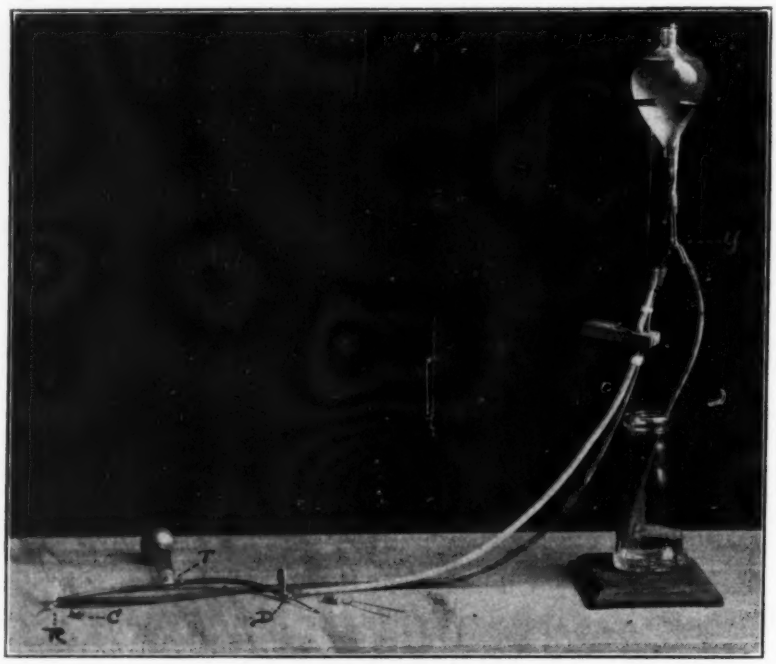

Fig. 1.

discarded Luer needle hubs with a drop of solder. By manipulating the clamp and the Bransford-Lewis syringe, the entire tube system is filled with solution and the air expelled. The veins are then entered using Lindeman cannula or some other large needle. The adapter caps are removed and the adapters are connected up to the needles. We usually use a Lindeman trocar needle for the donor and a platinum eighteen-gauge plain needle for the recipient. In transfusing no haste is required. The apparatus can be flushed frequently if desired, usually a short flush for each four or five syringe barrels of blood is sufficient. It can even be rested for a short time if filled with the citrate solution. Practically all the blood transfused is whole blood. A small portion of it may at times be citrated. In fact, I am not prepared to say whether or not whole blood has any advantages over properly citrated blood. At any rate, we make no effort to prevent a little citrate solution

getting into the recipient. This simple irrigating device can be applied to almost any whole blood apparatus by simply using needle adapters with side tubes. Very little chance exists for the recipient's blood entering the vein of the donor as the irrigating fluid enters just behind the donor's needle and flows toward the recipient. It may be that a slightly smaller syringe would work a little better. In fact, we have written to a manufacturer about this. We are now working on a valve to attach on the outside of the tubing which

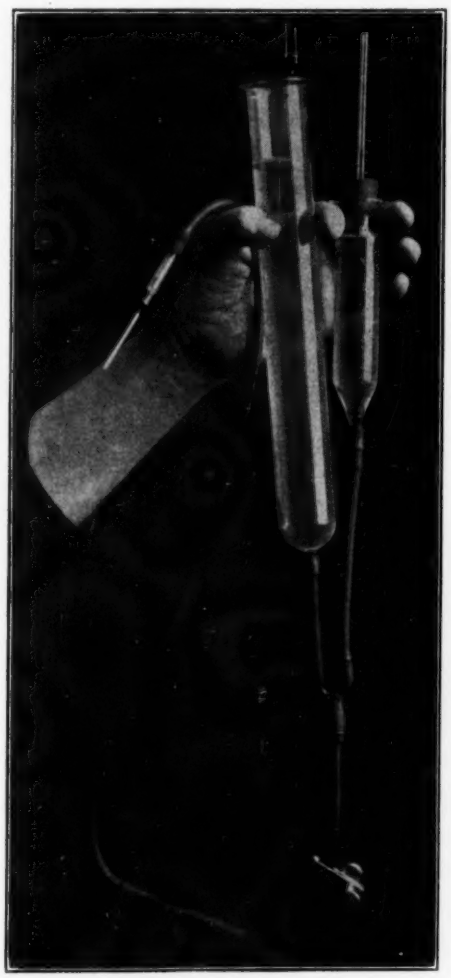

Fig. 2.

will span across the (T) tube, instead of using the fingers, but to date the fingers have the call.

Another apparatus which we use constantly and find very satisfactory is an infusion outfit for administering arsphenamine. This simple apparatus (Fig. 2) is made up of a 250 c.c. burette, the barrel of an Asepto syringe with a catheter tip, a rubber one-holed stopper with small piece of glass tubing, a Y-tube, and the conductor tubing. This will introduce first the isotonic salt solution, then the arsphenamine and follow with the salt solution again in a

semiautomatic manner which I will try to demonstrate. In this apparatus as in the transfusing apparatus we avoid stopcocks or valves in the tubing. The large burette is filled with salt solution which is allowed to expel all the air from the tubing and to back up into the small cylinder a short distance. The small cylinder is then clamped off and the arsphenamine, having been made up and alkalinized in the usual manner, is poured into the smaller cylinder after which the stopper with small glass tube is placed in the neck. The small glass tube is then made to just touch the arsphenamine solution. The clamp is now removed and the salt solution will back up to find a level in the small glass tube. Now the vein is entered and the solutions allowed to flow. The arsphenamine will not flow except for the very small amount represented in the small glass tube until the level of the lower end of the small tube is reached. After this the two tubes will fall equally, diluting the arsphenamine in the tubing as they flow. It has several advantages in that no stopcocks or other

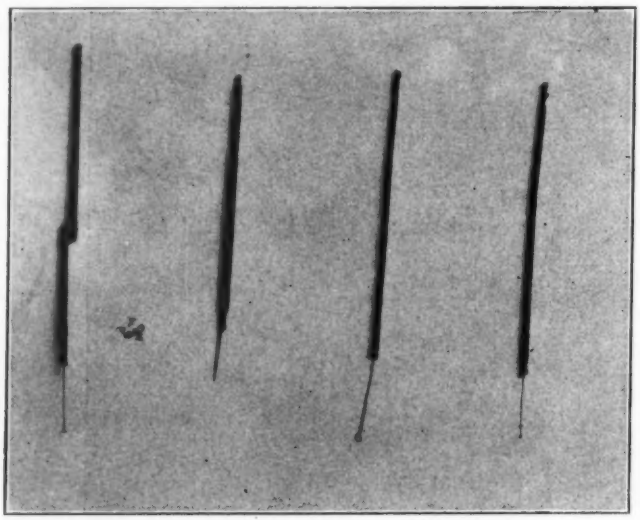

Fig. 3.

adjustments are required once it is started. The alkalinized arsphenamine can be kept quite cold and diluted only in distilled water until just before entering the vein. The salt solution can be brought well up to or slightly above body temperature, which will deliver at the needle practically an isotonic solution at body temperature.

Another small piece which has won a place in our laboratory is this style of handle for inoculating loops (Fig. 3), dissecting needles, etc. It is simply a short length of umbrella stay, the end of which is annealed by heating to a cherry red and the desired loop or needle clamped in with the blow of a small hammer. It has several advantages other than its cheapness in that it will not overheat with continued use, as will one which engaged the loop with a solid brass chuck or a solid glass rod. The loop cools rapidly and is sufficiently rigid, light and workable. By using a section near a joint a folding handle can be made which will carry well in a grip without continually bending off the needle.

URINARY PRESERVATIVES INCLUDING HEXAMETHYLENAMINE*

By Jeanette Allen Behre, Ph.D., and William Muhlberg, M.D.,
Cincinnati, Ohio

INTRODUCTION

THE work reported in this paper was undertaken with the intent of finding a satisfactory preservative for samples of urine which are to be analyzed for clinical or other practical purposes. There seems to be no preservative in general use at the present time which can be considered ideal for this purpose. The requirements for such a preservative may be summarized as follows:

1. That it should preserve the urine from bacterial decomposition and the development of moulds or other growths for considerable periods of time under adverse conditions.
2. That it should not interfere either positively or negatively with any of the physical, chemical or microscopic tests in ordinary use.
3. That it be readily soluble.
4. That it should not interfere to any marked extent with the normal reaction of the urine.
5. That it should be a solid.
6. That its cost be reasonable.

METHODS

With a view to finding a preservative which combined these conditions, we have investigated a number of varying substances. For the tests we used first normal urine, to which were added known amounts of glucose[1] and albumin.[2] The samples were kept in corked bottles in the incubator at 38° C. for periods of from four days to two weeks and were examined from time to time. These examinations included:

1. General macroscopic appearance of urine, odor, color, sediment, etc.
2. Sugar content as determined by the picric acid method of Benedict.[3]
3. Albumin by the sulphosalicylic acid method.[4]
4. Albumin by the nitric acid test.
5. Microscopic examination.
6. Reaction to litmus.

In further experiments the urine used was infected with samples already

*From the Laboratory of the Union Central Life Insurance Co.
Received for publication, July 30, 1925.

[1]J. T. Baker Chem. Co., Dextrose, C. P.

[2]Centrifuged sheep serum, diluted with 15 per cent NaCl.

[3]Benedict's Clinical Quantitative Test for Sugar in Urine, Hawk: Practical Physiological Chemistry, ed. 8, P. Blakiston's Son & Co., p. 665.

[4]Seven and one-half volumes of 3 per cent sulphosalicylic acid were added simultaneously to 2.5 volumes of urine and 2.5 volumes of each of a series of sheep serum standards corresponding to albumin concentrations of from 0.01 to 0.1 per cent.

containing bacteria, or with yeast. The preservatives which proved to be of most promise were also added to samples containing casts and red blood cells. The sodium nitroprusside test for acetone, and the Fehling test were also tried in connection with these.

PRESERVATIVES TRIED

The preservatives which we tried included the inorganic substances sodium fluoride, zinc chloride, boric acid and borax; and of organic substances, formaldehyde and toluene; certain phenols, such as thymol, tricresol, resorcinol and hexamethylresorcinol, benzoic and salicylic acids and their sodium salts, quinoline, camphor and menthol, acriflavine, chlorazene, and the so-called "Urofix" tablets.

The preservative which was decided upon as better than any of these, is a mixture of hexamethylenamine and salicylic acid. Before describing its advantages, however, a brief description of our results in connection with the other substances may be of interest.

Sodium fluoride is in most ways a very good preservative. It maintains the urine in a fresh condition for an almost unlimited period of time, in as low a concentration as 0.05 per cent and also does not interfere with sugar or albumin determinations at a concentration of 0.25 per cent. It produces a precipitate (probably calcium fluoride) in the urine, however, even in the lowest concentrations, which interferes with the microscopic examination and makes its use impracticable. Casts are apparently destroyed when sodium fluoride is added in any of the higher concentrations.

The combination of sodium fluoride with thymol, boric acid, sodium oxalate, sodium salicylate, sodium benzoate or zinc chloride did not improve the microscopic picture, nor could the sediment be washed free from the interfering precipitate with salt solutions, dilute acids or alkalis.

Potassium and ammonium fluoride yielded the same results.

Zinc chloride preserves the urine fairly well in amounts above 0.2 per cent and does not interfere with sugar reactions. It precipitates the albumin very appreciably, however, even when only 0.1 per cent is used, and it tends to disintegrate cells and form granular precipitates.

Boric acid does not interfere with the albumin reaction nor any of the microscopic constituents, but it does not preserve the urine under adverse conditions and interferes with the Benedict picric acid sugar determination in some positive way, giving sugar values which are too low. This is not due simply to the fact that the growth of yeast or sugar-destroying bacteria is not prevented, but it is a phenomenon which can be observed immediately after the boric acid is added, or in the presence of some efficient preservative, such as sodium fluoride or hexamethylenamine.

Borax has no recommendations, as it forms an alkaline urine, and even in connection with salicylic acid adds nothing as a preservative to the acid.

Formaldehyde is in many respects an ideal preservative. Concentrations of from 0.05 per cent to 0.1 per cent (depending upon the conditions and amount of infection) preserve the urine from bacteria and decomposition in

most cases without changing the microscopic picture or the albumin figures and without appreciably affecting the sugar value.

There are, however, some disadvantages to the use of formaldehyde as a preservative. In the first place, it is a liquid, which is the most important reason for discarding it. In larger amounts, it interferes with the sugar determinations which depend upon reduction. With the Benedict picric acid method it is found that urines containing 0.05 per cent of formaldehyde (1 drop "formalin" to 40 c.c. urine) show slightly more color than the urine without preservative and that this color increases with perfect gradation as the formaldehyde is increased. One-tenth per cent of formaldehyde in urines which are apparently sugar-free gives a color corresponding to about 0.1 per cent of sugar. With 0.5 per cent formaldehyde (about 10 drops of "formalin" per 40 c.c. urine) the color is very much deeper than the color corresponding to 0.1 per cent of sugar. The increase in color due to the smaller amounts of formaldehyde which would ordinarily be used for preserving the urine would not, however, be sufficient to interfere with the clinical determination of the sugar. In larger amounts, also (10 drops "formalin" to 40 c.c. urine) formaldehyde produces a sediment of globules or crystalline rosettes which interferes with the microscopic picture. Often complete disintegration of the cellular material takes place in the urine when these higher concentrations of formaldehyde are used.

Toluene is a fairly good preservative, but it is not as effective as formaldehyde, and is also a liquid.

Thymol was found to be an almost ideal preservative within certain rather restricted limits of concentration. These suitable concentrations are very low, from approximately 0.025 per cent to 0.1 per cent. When thymol is used in a percentage between these limits, the urine is beautifully preserved from bacterial or yeast invasions; no interference is found with the sugar determinations or with the albumin, as determined by the sulphosalicylic acid method, and the microscopic picture is found to be maintained in unusually good condition.

Larger amounts of thymol, however, form globular, gelatinous or chalky sediments. Also, even a very minute amount of thymol forms a small very compact ring with nitric acid, which in the presence of albumin is distinguishable from the albumin ring, lying below it and having a sharper appearance, but which might be mistaken for albumin in the latter's absence. Thymol has also been reported to interfere with the iodometric methods for acetone determination.

Even with these disadvantages, thymol was probably on the whole the second best preservative which we found.

Tricresol, Resorcinol and Hexamethylresorcinol are out of the question, since they all react in the picric acid sugar method, giving dark colors, and make turbid or milky or very dark colored urines with spongy or oily sediments.

Salicylic acid forms a moderately good preservative in amounts less than 0.1 per cent as far as sugar and microscopic examinations are concerned, but even at a concentration of 0.05 per cent it precipitates the albumin markedly, while in larger amounts it tends to increase the apparent sugar content and

to make the urine dark with a fine, dark sediment. It also gives the urine a strong acid reaction.

Sodium salicylate used in amounts which do not increase the specific gravity excessively does not preserve the urine adequately from bacteria or prevent glycolysis.

Sodium benzoate is open to the same objection.

Quinoline used in small amounts did not interfere with sugar or albumin, but it did not preserve the urine as adequately as many other preservatives, and its very peculiar and penetrating odor makes it highly undesirable for this use.

Camphor and menthol were not found to be adequate preservatives in moderate amounts and were discarded almost immediately.

Acriflavine (neutral acriflavine—Abbott) proved to be a fairly good urinary preservative even in such small amounts as 0.01 per cent, but the urine was so intensely colored by the dye that its use was out of the question.

Chlorazene (sodium-para-toluene-sulphochloramide—Dakin's antiseptic) was more promising. Used in concentrations of from 0.3 per cent to 0.75 per cent it preserved the sugar and albumin well, and did not interfere with the microscopic picture. The urine was kept clear and light, with very slight sediment. Smaller amounts, however, did not preserve the urine, while larger amounts bleached it to a chalky or completely colorless condition with no sediment. Casts were usually injured, even when the smaller concentrations of chlorazene were used.

The urofix tablets, put out by Wolf, were given a careful consideration, especially in connection with the hexamethylenamine-salicylic acid mixture which we chose as being the best preservative. It was found that one urofix tablet (supposed to be adequate for the preservation of 2 oz.) would preserve 40 c.c. of urine moderately well under favorable conditions, but that it often failed to keep the urine in good condition when bacteria were introduced. Even under the best conditions one tablet produced a brownish sediment, usually including small dark granules, which appeared in the microscopic picture. Two urofix tablets, which preserved the urine from infection in most cases, produced much more abundant sediments, which often interfered with the microscopic picture. A noticeable amount of albumin was also often precipitated by the urofix tablets.

HEXAMETHYLENAMINE AND SALICYLIC ACID

The preservative which was found to be the best, almost without any question, is a combination of hexamethylenetetramine (urotropin) with salicylic acid. As is well known, hexamethylenamine liberates formaldehyde and ammonia in acid solution, and it seemed that theoretically this would make an ideal preservative which would have all of the advantages of formaldehyde with the added advantage of being in a solid form. This expectation appeared to be fully justified. In many cases the new preservative seemed to maintain the normal condition of the urine more satisfactorily than did formaldehyde. The concentrations which were chosen as being the best were 0.3 per cent hexamethylenamine and 0.2 per cent salicylic acid.

Hexamethylenamine alone was found to preserve acid urines moderately well, but, with the salicylic acid added, even alkaline urines and urines badly infected with bacteria are remarkably well preserved. Salicylic acid was chosen as being a good preservative in itself. Benzoic acid did not give quite such good results. The disadvantages found in connection with using salicylic acid alone were all obviated when it was combined with the base. The reaction of the urine with the preservative added is amphoteric to litmus, sometimes more acid, sometimes more alkaline, but always at least slightly amphoteric. The amount of salicylic acid which we decided to use is such that even urines made distinctly alkaline with sodium carbonate turned blue litmus red when the preservative had dissolved in them, and even these urines were well preserved.

With the new preservative sugar and albumin concentrations are maintained, and there is no appreciable interference with any of the tests, including Fehling's and the nitroprusside and iodoform tests for acetone. Casts and red cells are preserved. Yeast does not form gas with the preservative alone in sugar-free urine, and fermentation of sugar in the urine is repressed though not entirely inhibited in the presence of the preservative. Two sets of normal urine were kept with this preservative for twelve weeks without showing any change in sugar or albumin content and giving an almost perfect microscopic picture at the end of the time, with epithelial cells intact and no bacteria. Many sets were kept in excellent condition for at least six weeks.[5]

That formaldehyde really is formed in the urine from this combination is unquestionable. Water solutions of the mixture in the same concentration give a faint though characteristic odor of formaldehyde after a few days, and urines containing it have the same sweetish almost unmistakable odor as do urines which are preserved with very small amounts of formaldehyde. With larger amounts of the mixture, the odor becomes more characteristically that of formaldehyde.

Furthermore, when the preservative is added to sugar-free urine, and the picric acid test performed at intervals, it is found that a very slight increase in the faint color given by the urine alone occurs from day to day. When twice the amount of preservative is used, this color, after twenty-four hours, matches the color given by 0.05 per cent of formaldehyde in the same urine. This increase in color, like that given by formaldehyde itself, is noticeable only in urines of which the sugar content is very low, and it is not great enough to make any appreciable difference in the results of the sugar determination. The formaldehyde is evidently produced gradually as the reaction goes on.

It is interesting to note also that salicylic acid in concentrations exceeding its solubility in pure water is dissolved at once when sufficient hexamethylenamine is added, and that when less hexamethylenamine is added the solid salicylic acid, which is not at first dissolved in the presence of the base, gradually does go into solution, evidently being used up in the course of the reaction.

It seems probable that the salicylic acid acts as a preservative of the urine during the first period while the formaldehyde is being produced.

It would be of considerable advantage for routine work if the preservative

[5]Since the completion of this investigation the new preservative has been in use for four months in the Union Central Life Insurance Company with entirely satisfactory results.

mixture could be put into tablet form. We have not yet found an entirely satisfactory way of doing this, though we hope to do so eventually. It has been found perfectly satisfactory, however, even when routine work is being done on a large scale, to use an intimate mixture containing three parts of powdered hexamethylenamine and two parts of salicylic acid. This is added to the urine in the proportion of 50 mg. to 10 c.c. of urine.

SUMMARY

A brief description is given of the advantages and disadvantages found in connection with a number of substances which were tried and discarded as urinary preservatives.

A preservative which seems to fulfill the requirements for practical urinary analysis is described. This consists of a mixture of hexamethylenetetramine and salicylic acid in the ratio of three parts to two. Fifty mg. of the mixture are used for the preservation of every 10 c.c. of urine.

A VALVELESS ADAPTER FOR A TWO-WAY SYRINGE*

By Paul G. Weston, M.D., Jamestown, N. Y.

FOR the purpose of dispensing with valves in a two-way syringe, an adapter using the burette stopcock principle was made. The projections *A A* are adapters for a Luer socket and *B*, for a Luer syringe nozzle. *C* is a small screw holding the two parts together.

For use the piston is withdrawn and the syringe barrel fills through the adapter on the right. The syringe is then given a half turn and its contents

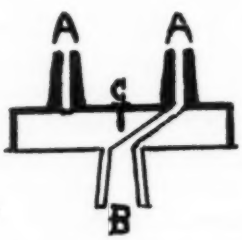

Fig. 1.

ejected through the adapter on the left. The process is repeated until the required amount of fluid is injected or, in the case of aspiration, until the desired amount is withdrawn. A small stud, not shown in the diagram, prevents more than a half turn being made.

*Received for publication, December 7, 1925.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Snell, A. M., Greene, C. H., and Rowntree, L. G.: Diseases of the Liver. II. A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice. Arch. Int. Med., August, 1925, xxxvi, 273.

In the fructose tolerance test given to dogs there was a definite reduction in tolerance, a rise of 40 mg. per cent above the normal being the rule.

There was a definite fall in the nonprotein nitrogen following the development of jaundice, the decrease involving both the relative and absolute urea values The values, however, as a rule, were within the lower limits of normal.

The changes in the bilirubin content of the serum are characteristic and constitute a test of definite value. The findings closely parallel those of the tetrachlor test. Dye retention first appears with the development of bilirubinemia.

The coagulation time is prolonged though the fibrin content was increased and serum calcium unchanged.

Greene, C. H., McVickar, C. S., Rowntree, L. G., and Walters, W.: Diseases of the Liver. III. A Comparative Study of Certain Tests for Hepatic Function in Patients with Obstructive Jaundice. Arch. Int. Med., September, 1925, xxxvi, 418.

The following tests were studied: fructose tolerance, blood-sugar level; nitrogen partition; bile pigments in the blood; and phenoltetrachlorphthalein test.

The greater number of tests studied failed to show changes sufficiently significant to be of clinical value. The most valuable clinically were the studies of the serum bilirubin content, in which an increase occurs before there is demonstrable jaundice, and of the tetrachlor test, which closely parallels the pigment curve.

Greene, C. H., McVickar, C. S., Walters, W., and Rowntree, L. G.: Diseases of the Liver. IV. Functional Tests in Cases of Carcinoma of the Liver and Biliary Tract. Arch. Int. Med., October, 1925, xxxvi, 542.

Report of various functional tests in malignancy, the cases being grouped as follows:

I. Metastatic carcinoma of the liver associated with jaundice, 14.

II. Metastatic carcinoma of the liver without jaundice, 18.

III. Intraabdominal carcinoma without demonstrable involvement of the liver, 17.

In eight cases in Group I fructose tolerance was lowered; this also occurred in one case in Group II and in two cases in Group III. Because of the irregularity in the results and the frequent association of conditions affecting the carbohydrate mechanism, the authors regard this test as of little clinical value.

There were no significant changes in the blood-sugar level, nor were any changes found in the nonprotein nitrogen of the blood which could be attributed to the carcinomatous process. The most useful clinical tests were the determination of the serum bilirubin and the tetrachlorphthalein excretion.

In Group I the icterus index was increased, and a direct van den Bergh reaction was obtained.

In Group II a zone of latent jaundice was obtained in three cases, normal values being found in Group III.

The findings were comparable to those already reported and of definite clinical value.

Tetrachlorphthalein readings:

In Group I the maximal reading in one hour was 51 per cent, the average being 21 per cent.

In Group II the maximal reading was 28 per cent, definite retention occurring in eleven of the eighteen cases. A normal reading was noted in five of these cases.

In Group III three cases showed slight retention, two questionable retention, and the others were normal.

The authors believe that the serum bilirubin and tetrachlor tests are the only tests of those studied which are of cinical value in the early diagnosis of carcinoma of the liver and biliary tract.

Larson, W. P., and Halvorson, W. O.: The Effect of Concentration upon the Neutralization of Toxin by Sodium Ricinoleate. Proc. Soc. Exper. Biol. and Med., 1924, xxi, 278.

Continuing their studies of the action of surface depressants on bacterial toxins, the authors present the results of an attempt to show the effect of concentration of both soap and toxin upon the efficiency of detoxification.

Their experiments indicate that concentrations of soap below 5 per cent do not completely detoxify and that equilibrium is reached within a comparatively short time.

Other experiments show that the action of soap on toxin is a reversible one, that the toxin is not destroyed, and that the neutralization of toxin by sodium ricinoleate is an adsorption phenomenon.

Larson, W. P., and Colby, W.: Immunization Against Scarlet Fever Using Sodium Ricinoleate as a Detoxifying Agent. Proc. Exper. Biol. and Med., 1925, xxii, 549.

Scarlet fever toxin, the potency of which was 15,000 skin test doses per c.c., was mixed with a sodium ricinoleate solution in amounts sufficient to make a final soap concentration of 1 per cent, and this mixture was used to immunize human beings.

As the toxin had been completely neutralized it was found that 1000 skin test doses could be given to children with entire safety. Using such a "neutralized" toxin, 148 children from one to sixteen years of age who gave positive Dick tests, were injected with varying amounts of the soap-toxin mixture and, after one such injection, Dick retested in five to twenty-one days.

Three weeks after this single injection, 96 per cent were Dick negative. In 15 per cent there was a slight local reaction appearing within twelve hours and gone within thirty-six hours.

A group of adults were given 2000 skin test doses of the soap-toxin mixture without any reaction, 37.5 per cent being Dick negative in seventeen days. Larger doses, 4000 units, are recommended for adults. The soap-toxin mixture must be perfectly clear, should stand at room temperature for four hours to allow an equilibrium to become established, and should be kept in hard glass containers.

Larson, W. P., Howard, E. W., and Eder, H.: Antidiphtheretic Immunization Using Sodium Ricinoleate as a Detoxifying Agent. Proc. Soc. Exper. Biol. and Med., 1925, xxii, 552.

The ability of sodium ricinoleate to detoxify bacterial toxins has been pointed out in previous communications by Larson and his associates, and the present paper reports the results of studies conducted to determine if this property could be utilized in the active immunization of children and adults against diphtheria, using such a detoxified toxin.

A quantity of diphtheria toxin was mixed with a 1 per cent solution of sodium ricinoleate so that 1 c.c. of the mixture contained 0.125 L + toxin, and 1 c.c. of this mixture was injected.

Neither local nor general reactions occurred in 149 children and adults so treated. Of those so injected, 69 were retested within six weeks, 50.2 per cent giving a negative Schick reaction.

The method is reported as feasible for practical use and very effective, a majority of those so treated giving a negative Schick reaction within four weeks after injection of one dose.

Larson, W. P., Halvorson, H. O., Evans, W. D. and Green, R. G.: The Effect of Surface Tension Depressants Upon Bacterial Toxins. Colloid Symposium Monograph, New York, J. J. Little and Ives Co.

The authors report the effects of sodium soaps of various fatty acids on bacterial growth and bacterial toxins.

Soaps which form colloidal solutions do not appear to adsorb upon bacteria or their toxins.

Sodium ricinoleate, which seems to form a true solution, has a marked effect upon bacterial toxins in that, without any change in their antigenic effects, the toxins no longer produce toxic effects when injected into animals. This surface depressant action completely destroys the toxic action of tetanic, diphtheritic, and scarlatinal toxins so that several hundred fatal doses may be injected without injurious effect.

Injection of such detoxified toxins is followed by the production of immune bodies against the normal toxins and such large doses may be used that immunization occurs rapidly.

On the basis of these results soap-detoxified toxins have been used in the immunization of human beings against diphtheric and scarlatinal streptococcic toxins.

It is imperative that the soap must form a clear solution, that no foreign particles be present, and that the solutions remain perfectly clear. It has also been found necessary to allow the mixture to stand for some time (four hours at room temperature) to permit an equilibrium to be established.

The action of the soap appears to be an adsorption phenomenon. The concentration of both soap and toxin is of importance.

Experiments are cited which indicate that a toxic mixture may be made nontoxic by diluting the toxin and keeping the concentration of soap constant, or by increasing the soap concentration without changing the concentration of the toxin.

The assumption is that in free toxin preparations the toxin exists in colloidal aggregates capable of dispersion and that to secure complete protection the aggregates must be dispersed and a layer of soap adsorbed over the entire surface of the toxin molecule.

Dispersion is brought about either by adding a sufficiently concentrated soap solution, or by diluting the toxin.

Concentration of the toxin, rather than the dosage, appears to be the prime factor in controlling the amount of antibody formed.

Black, J. H., and Moore, M. C.: Pollen Therapy with Protein-free Extracts. Jour. Am. Med. Assn., January 30, 1926, lxxxvi, 5:324.

Evidence is presented that the activity of pollen extracts is unimpaired by the removal of the protein constituents and that standardization by nitrogen content of complement fixation does not indicate the content of active substance.

Scudder, S. A: A Comparative Study of the Value of Stained Smears and Cultures in the Diagnosis of Gonorrheal Vulvovaginitis. Jour. Urol., November, 1925, xiv 5, 429.

In a carefully controlled study of twenty-four cases, several modifications of Gram's stain were used, the most satisfactory being the akaline method described by Burke, using 95 per cent alcohol as a decolorizer. The exact details of the technic used by the author are not given in the original paper.

Cultures were made on a modification of the Thalmann medium (veal-infusion-glycerol agar to which urine and Grubler's Iodgrun were added) prepared as described by Torrey (Jour. Infect. Dis., 1922, xxxi, 125).

Semisolid agar to which a few drops (less than 0.5 c.c.) of ascitic fluid, or a good grade of horse plasma without preservative, were added was found to be an excellent emergency medium.

Organisms culturally and morphologically similar but not gonococci were not encountered.

The fermentation test is the most reliable means of differentiation.

The stained smear, when carefully made, checked and controlled, is a reliable method of diagnosis.

The paper contains much informative data, but the methods used are only indefinitely described, reference to the original papers mentioned being necessary to ascertain the exact technic.

Reith, A. F.: Bacteria in Muscular Tissues and Blood of Apparently Normal Animals, A Preliminary Report. Jour. Am. Med. Assn., January 30, 1926, lxxxvi, 5, 325.

Cultures of the muscular tissues of slaughtered dogs show the presence of bacteria in 77 per cent of 216 samples; 57 per cent contained anaerobes.

In healthy, live hogs, rabbits, and guinea pigs 83 per cent of 108 samples contained bacteria, anaerobes being found in 49 per cent.

Blood cultures from healthy animals were positive in 67 per cent of 18 samples, anaerobes being found in 44 per cent.

The investigations were part of a study of meat spoilage.

Jackson, G.: Crystal Violet and Erythrosin in Plant Anatomy. Stain Technology, January, 1926, xii, 1:33.

Satisfactory differentiation between liquefied and nonliquefied cells is obtained by the following technic:

1. Remove paraffin with xylol.
2. Remove xylol with absolute alcohol.
3. Ninety-five per cent alcohol.
4. Crystal Violet, 1 per cent in distilled water, fifteen minutes.
5. Rinse quickly in water.
6. Dehydrate quickly but thoroughly in 95 per cent and absolute alcohol.
7. Erythrosin, saturated solution in clove oil, one to five minutes.
8. Absolute alcohol and xylol, equal parts, one to two minutes.
9. Xylol.
10. Mount in balsam.

Genung, Eliz. F.: Basic Fuchsin as an Indicator in Endo's Medium. Stain Technology, January, 1926, i, No. 1, 41.

A method is described for preparing uniform solutions for Endo's medium.

The stock solution of fuchsin is made by dissolving 10 grams in 100 c.c. of 95 per cent alcohol. Dilutions are made in test tubes by adding to 1 c.c. of the stock solution, 1 c.c. (1:1), 2 c.c. (1:2), 3 c.c. (1:3), 4 c.c. (1:4), and 5 c.c. (1:5) of 95 per cent alcohol. To 10 c.c. of 2.5 per cent sodium sulphite solution is added 0.5 c.c. of each of the above dilutions. The tubes are allowed to stand and the lowest dilution of fuchsin, which is decolorized promptly, is used in the preparation of Endo's medium. The reaction of the agar is best placed at PH 7.4.

When a new lot of dye is used it should be tested with colon, aerogenes, and typhoid bacilli.

Harned, B. K.: The Sugar Content of Blood. Jour. Biol. Chem., October, 1925, xlv, 3, 555.

The Folin-Wu blood-sugar technic applied to blood filtrates after mercuric nitrate precipitation gives a filtrate relatively free from nonsugar interfering components; the results are from 17 to 25 per cent lower than those obtained with the Folin-Wu method and are in accord with Benedict's latest method.

Method.—Mercuric nitrate reagent: 348 gm. of mercuric nitrate are shaken in a liter flask with 900 c.c. of water for ten to fifteen minutes. Concentrated nitric acid is then added, a few c.c. at a time, and later drop by drop until the mercuric nitrate is dissolved. The solution is then made up to one liter.

1. In an Erlenmeyer flask dilute 1 volume of blood with 8 volumes of water.
2. While rotating the mixture add 1 volume of mercuric nitrate mixture, a little at a time. The proteins are precipitated in a jelly-like mass.
3. Stopper the flask and shake vigorously to break up the precipitate.

4. Filter.

5. Add solid sodium bicarbonate to the filtrate until effervescence ceases and the reaction is faintly alkaline to litmus.

6. Remove the resultant precipitate by filtration through a double filter.

7. Add dried and finely powdered potassium bisulphate until a drop of the mixture gives with tropeolin 00 on a spot plate the intermediate color shade.

8. Remove traces of mercury from the solution by precipitation with hydrogen sulphide which is first passed through water.

9. After filtering off the mercuric sulphide, blow excess hydrogen sulphide out of the solution by a current of air which first passes through a wash bottle.

The solution is then carried through the usual Folin-Wu technic, the standard being brought to the same reaction as the unknown by the addition of potassium bisulphate.

The method is not advocated as a routine procedure but is recommended as a technic which can be relied upon for the determination of the absolute blood-sugar level.

Holmes, W. C.: The Oxidation Products of Methylene Blue. Stain Technology, January, 1926, i, 1, 17.

The preparation of a new product, Azure C, which has valuable properties as a nuclear and bacterial stain in tissue and which may be substituted for Azure A in the MacNeal tetrachrome blood stain is described.

Dissolve 2.5 grams of methylene blue in 200 c.c. of water and bring to a boil. While boiling add a hot solution containing 5 c.c. of concentrated HCl (36 per cent) in 100 c.c. of water and 2.5 grams of potassium bichromate. Place in the autoclave for one hour at 115 to 120° C.

The resultant product has been termed Azure C. As a tissue and bacterial stain it is used as follows:

1. Xylol—3 min.
2. Absolute alcohol—3 min.
3. Ninety-five per cent alcohol—3 min.
4. Water—3 min.
5. Stain in the following for 5 minutes:

Azure C	1.5 gm.
Distilled water	100.0 c.c.

Note.—As a PH of 7.5 or greater is necessary for consistent results, 1 per cent of disodium hydrogen phosphate has been added to the azure. This has been found to be an essential, as in commercial production it is found that there often remains in the dry dye quite a little residual acid which must be neutralized. The presence of this phosphate does not interfere with the utility of the azure in other combinations.

6. Absolute methyl alcohol, five to ten seconds.

Note.—This is an important step preparatory to differentiation. The sections should be left in the methyl alcohol until no more free blue is apparently washed out; this is ordinarily accomplished in a few seconds. In case the final result shows a lack of sharpness of nuclear and bacterial staining, it is probably due to improper treatment in this step.

7. Differentiate and counterstain in:

Eosin Y and Orange II mixture	0.050 gm.
Glacial acetic acid	1.0 c.c.
Absolute ethyl alcohol	99.0 c.c.

Note.—Carry this step until no more free blue is washed out of the section, generally thirty to forty seconds. With steps 5 and 6 properly accomplished, it is difficult to overdifferentiate, and little fear need be entertained in that direction. The eosin Y and orange II mixture referred to, is a mixture of eosin Y (C.1.768) and orange II (acid orange Y, C.1.151).

8. Absolute alcohol, 1 to 2 min.
9. Absolute alcohol, 1 to 2 min.
10. Xylol, 1 to 2 min.
11. Xylol, 1 to 2 min.
12. Xylol Balsam.

In practice the writers employ Coplin jars in this staining technic, using the staining solution and alcohols for a considerable period of time. There seems to be little deterioration in the solutions, some apparent discoloration not interfering with consistent results.

The stain may be used after formalin or Kaiserling fixation.

Ackman, F. D.: The Relation Between Gastric Acidity and the Hydrogen Concentration of the Urine with a Study of the Effect of Histamine. Canadian Med. Assn. Jour., 1925, xv, 1099.

The method of Henderson and Palmer (Jour. Biol. Chem., 1912, xiii, 393) was used for the determination of the urinary PH, except that 2 per cent aqueous alizarin was used where the PH was less than 7.4, and 1 per cent alcoholic phenolphthalein for PH of 8.0 or over.

The fractional method of gastric analysis was used in this investigation.

Studies were made of 67 normal subjects, 31 cases of achlorhydria from various causes, and 15 cases of hyperchlorhydria.

Conclusions:

1. The occurrence of the "alkaline tide" is corroborated, the lowest urinary acidity occurring usually three hours after a meal.

2. A relatively high acidity occurring at 9 A.M., 1 P.M. and 5 P.M. was not explained.

3. In cases of normal gastric acidity, the degree of urinary acidity varies inversely with the gastric acidity.

4. In hyperchlorhydria the daily urine tends slowly toward alkalinity; in achlorhydria the alkaline tide is wanting.

5. A high carbohydrate diet produces little or no alkaline tide; a high protein diet produces a conspicuous tide, due to differences in the gastric stimulation.

6. In starvation the alkaline tide is eliminated.

7. A meal always produces a change in the urinary reaction in normal individuals.

8. The evidence supports the gastric origin of the alkaline tide, and, when gastric analysis is impossible, the hourly PH may furnish information of clinical value concerning the secretory ability of the stomach.

9. Histamine, subcutaneously, stimulates the gastric secretion.

Hogue, M. J.: Staining Protozoa with Janus Green B. Stain Technology, January, 1926, i, 1:35.

One drop of 0.5 per cent aqueous solution to 10 to 20 drops normal saline (for parasitic) or water (for free living protozoa) added directly to a drop of culture will give a satisfactory flagella stain.

A dilution of 1:20 of 0.5 aqueous solution of the dye, will give vital staining, the protozoa finally becoming a deep pink as they dye.

Stadie, W. C., and Ross, E. C.: A Micro Method for the Determination of Base in Blood and Serum and Other Biological Materials. Jour. Biol. Chem., October, 1925, xlv, 3:735.

A method eliminating various sources of error.

Materials needed:

Standardized N/50 NaOH.

Benzidin hydrochloride solution.

Benzidin ---------- 4 gm.

N HCl ----------45 c.c.

Dissolve and make up to 250 c.c. Filter if necessary. This solution is standardized by titrating 2 c.c. against N/50 NaOH, using phenol red. It keeps indefinitely, requiring occasional filtration. It is best to restandardize about once a week, although as a rule the titer is unchanged over two to four weeks. Its equivalent concentration is about:

Benzidin hydrochloride----------	175	m.-Eq. per liter.
Excess HCl ----------	5	" " "
Total ----------	180	" " "

10 c.c. burettes graduated in 0.05 c.c.

50 c.c. silica beakers.

METHOD

Place 1 c.c. of serum or an aliquot of a trichloracetic acid filtrate of whole blood or cells (see below) corresponding to about 1 c.c. of blood in a 50 c.c. silica beaker. Add 0.5 c.c. of concentrated sulphuric acid and evaporate at low heat (about 90° C.) on a sand bath until practically all the water is driven off. Add 1 c.c. of concentrated nitric acid and continue heating, raising the flame from time to time. Add 1 c.c. of nitric acid occasionally to aid in the oxidation of organic matter. When the full heat of a triple burner has been reached and a clear white ash obtained, transfer to a triangle and heat fifteen minutes at bright red heat, allowing the flame to play over the entire outside of the beaker. This as a rule suffices to drive off all excess of sulphuric acid. Let the beaker cool to room temperature; add 15 c.c. of water. If preferred, the ash may be dissolved and quantitatively transferred to a 20 c.c. flask for precipitation. The ash readily dissolves with a little stirring. To be certain that no excess of sulphuric acid is present, add a drop of phenol red and titrate with N/50 NaOH. Rarely is more than 0.05 c.c. required. This amount may be neglected, but if more is necessary a correction must be made for it in the calculation. Add 2 c.c. of standardized benzidin hydrochloride and filter through a small dry quantitative filter into a dry Erlenmeyer flask. Titrate 15 c.c. of the filtrate with N/50 NaOH, adding more indicator if necessary.

Calculation of Milli-Equivalents of Base per Liter of Blood or Serum. For 1 c.c. of blood or serum analyzed as above we have,

$$\text{m.-Eq. base per liter} = (\text{titer of 2 c.c. of benzidin HCl} - \frac{17}{15}\ \text{titer of 15 c.c. of filtrate})$$

$$= \text{c.c. N/50 NaOH} \times 0.02 \times 1000.$$

If the amounts given above are varied as occasion may direct, we have for N/50 NaOH.

$$\text{m.-Eq. of base per liter or per kilo} = 0.02\ (\text{titer of x. c.c. benzidin HCl} - \frac{\text{total volume}}{\text{aliquot}} \times \text{titer aliquot}) \div \text{amount of material in liters or kilos.}$$

If more than 0.05 c.c. of N/50 alkali are required for the end-point of the ignited ash subtract from the titer of 2 c.c. of benzidin HCl in above formula. Allowance, of course, is made in the "total volume" for the added volume which is increased by the added alkali.

Calculation of Base Bound to Phosphate. As a rule the base in blood bound as phosphate is less than two milli-equivalents per liter and may for most purposes be neglected. Since the base as phosphate is not determined in this method, it must be separately determined when the phosphate concentration is a significant part of the total. The micro-methods of Tisdall (1922) and Briggs (1924) are available. To the base as determined above add one milli-equivalent for each milli-equivalent of phosphorus. In the ignited ash, the phosphate is present as metaphosphate BPO_3 binding one equivalent of base per mol of phosphorus. If desired the phosphates may be removed by the method described below and the total base (including phosphate base) determined.

Preparation of Trichloracetic Acid Filtrate of Whole Blood or Red Cells. Place 3 c.c. of whole blood (or about 3 gm. accurately weighed of red cells) in a 50 c.c. volumetric flask. Add about 20 c.c. of water, and when hemolysis is complete add slowly, with vigorous shaking, 25 c.c. of a ten per cent trichloracetic acid solution. Make up to volume and filter. Yield about 45 c.c. Twenty c.c. of the filtrate are ashed as above.

When the ratio in equivalents of phosphate to base exceeds 10-150, the removal of phosphates becomes necessary and is achieved by the following method:

Technic for the Removal of Phosphate. The material (urine, tissue, gastric contents, feces, etc.) in an amount sufficient to give about 0.100 to 0.200 milli-equivalent of base is digested in a silica beaker with sulphuric and nitric acids until the organic matter is oxidized. Do not ignite to complete dryness at any time. Transfer to a 25 c.c. volumetric flask, using about 15 c.c. of H_2O; add a few drops of phenol red; neutralize with 4 N NH_4OH (1:4 concentrated NH_4OH). Render just acid with a drop or two of normal H_2SO_4. Add 0.1 N ferric ammonium sulphate (0.033 m.) using about 6 equivalents of iron for every equivalent of phosphate. In practice 1 c.c. of 0.1 N ferric alum will completely precipitate up to 0.020 milli-equivalent of phosphate in the sample. Add 0.1 N NH_4OH just to a full red to the indicator and filter. Transfer 20 c.c. of the filtrate to a silica beaker or platinum dish, add a drop of concentrated H_2SO_4, evaporate, and

ignite, continuing the analysis as outlined under method for blood. In the calculation, of course, due allowance is made for the aliquot taken.

Wohlbach, S. B., and Howe, P. R.: Intercellular Substance in Experimental Scorbutus. Arch. Path. and Lab. Med., January, 1926, i, 1:1.

In a paper with sixteen illustrations (microphotographs) a careful and extensive study is reported tending to demonstrate that scorbutus represents an inability of the supporting tissues to produce and maintain intercellular substances.

It is suggested that this failure is due to the absence of an agent common to all supporting tissues, which is responsible for the setting or jelling of a liquid product. Antiscorbutic substance is liberated in the destruction of tissues. The paper does not lend itself well to abstraction and should be read in the original.

Lippincott, L. S.: A Technic for Preparing Frozen Sections. Stain Technology, January, 1926, i, 1:39.

The method described has been very satisfactory.

1. Formalin 10 per cent overnight.
2. Place in distilled (not tap) water, freeze in a drop of distilled water and cut.
3. Transfer to distilled water and thence to a slide.
4. Drain and cover with 95 per cent alcohol.
5. Blot carefully on cover with very thin celloidin in equal parts of alcohol and ether.
6. Drain off celloidin and allow to set in air but do not dry.
7. Distilled water for two minutes.
8. Ripened Delafield's hematoxylin.
9. Two washings in clear distilled water.
10. 0.5 per cent equeous eosin two to five minutes.
11. 95 per cent alcohol till no further stain is removed (five minutes).
12. Drain off alcohol and flood with beechwood creosote. (Ten to fifteen minutes.)
13. Blot, mount in balsam, and examine.

Kalodny, A.: Hypernephroma of the Thyroid. Arch. Path. and Lab. Med., January, 1926, i, 1:37.

The second case in the world's literature is reported and described

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Clinical Researches in Abdominal Disease**

THE author first emphasizes the value and importance of clinical research, as contrasted and correlated with pure laboratory research. "It is within the scope of the clinician to test with thoroughness the results achieved by work in the laboratory. It is a tribute to the clinician that the laboratory worker eagerly acclaims clinical results when they appear to support his experimental findings but quarrels with their accuracy when they do not confirm his views. It is also within the scope of clinical research to try various empirical methods and to furnish the results to the laboratory for explanation and elaboration. The clinical researcher must also record and compare clinical facts with the object of determining some questions which are inaccessible to laboratory methods of study."

The author discusses in detail the functions of the parietal peritoneum in localization of abdominal pain and zones of hyperesthesia or hyperalgesia, commonly known as Head's zones. Contrary to the usual statement, Dr. Cope finds that the location of the shoulder pains, whether anterior or posterior, aids in the localization of diaphragmatic irritation. The symptom on the other hand does not enable one to determine whether the pathology is above or below the diaphragm. Cope finds hyperesthesia in 59 per cent of his cases of acute appendicitis and in 47 per cent of all cases coming under the general category of the acute abdomen. The hyperesthesia may occasionally be left-sided in the former. The location of the hyperesthesia in the latter is not of distinct value in localizing the acute intra-abdominal lesion.

The Emotional Life of the Child†

THE experimental investigations of Helga Eng of Oslo into the emotional life of the child as compared with that of the adult have been translated by George H. Morrison. While the field of the author's experimental work is much more restricted than one would anticipate from the title, the results and conclusions are of unusual interest. She has aroused the various emotional responses in children and adults, following which she has traced changes in the pulse volume, arm volume, heart rate and the respiratory rate and depth. The author finds that spontaneous displeasure or depression curves are much less frequent in children than in adults, while spontaneous pleasure

*By Zachary Cope, B.A., M.D., M.S. (Lond.), F.R.C.S. (Eng.). Cloth. Pp. 148. Price $4.00. Humphrey Milford. Oxford Univ. Press. 1925.

†Experimental Investigations into the Emotional Life of the Child compared with that of the Adult. By Helda Eng, Oslo. Translated by George H. Morrison, M.B. (Edin.). Cloth. Pp. 243. Humphrey Milford. Oxford Univ. Press. 1925.

curves as well as pleasure curves resulting from external stimuli are more frequent. The work covers a wide range of emotions which cannot be reviewed in the space at our disposal.

On the basis of experimental evidence the author takes exception to the theory of James that we do not cry because we are sad but we are sad because we cry. She believes that the psychic processes and reactions more nearly control the somatic function than the reverse.

Among those psychologists who have made any intensive studies of the emotions with this or similar methods, we find nearly as many hypothetical conclusions as there are investigators. While the work probably will be subject to some criticism as other methods of study are developed, it now holds a high place in the literature on the subject.

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*Physical Chemistry in Biology and Medicine**

THE advancement of science is partly dependent upon the application of the discoveries in one branch to other branches. This volume is intended to indicate the application of recent and advanced work in physical chemistry to biology and medicine. It is not in any sense a textbook on the subjects covered but is intended for the research worker in biology and medicine, to whom it should prove a useful and rather comprehensive reference.

Within its fifteen chapters a résumé of all the recent work in this field is clearly presented.

The book is well arranged and well printed, and, though the subject is complex, the style is clear and readable.

Postmortem Appearances†

MORGAGNI, the founder of modern pathology, interested himself in gross pathologic lesions. With the passage of time interest has centered more and more in microscopic appearances, and this phase of the study has been stressed in the medical curriculum to such an extent that not infrequently one finds students who have the impression that pathology is always a question of microscopic appearance. The medical student learns to diagnose abnormal tissue changes with considerable accuracy, but we would find not infrequently that if he were handed a whole organ on which to make a gross diagnosis he would fail lamentably.

This weakness in our teaching system, which fortunately is being rapidly overcome, is an important one because the surgeon from the nature of his work must be a thoroughly trained gross pathologist. He must be able to recognize and interpret gross changes immediately, in so far as possible, during the operation, so that he may best decide upon the proper operative procedure.

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*Physical Chemistry in Biology and Medicine. By J. F. McClendon, Assistant Professor of Physiologic Chemistry, and Grace Medes, Assistant Professor of Physiologic Chemistry, University of Minnesota, Medical School. Cloth. 34 illustrations. 425 pages. Price $4.50. W. B. Saunders Company.

†Postmortem Appearances. By Joan M. Ross, M.B. B.S., (Lond.) M.R.C.S., L.R.C.P., with preface by E. H. Kettle, M.D. Cloth. Pp. 216. Price $2.50. Humphrey Milford. Oxford Univ. Press. 1925.

To a certain extent this little handbook fulfils the requirements of a reference manual on gross pathology. The reviewer feels that while it is laudable, indeed essential, to describe the changes in remote organs affected during the process of a local disease, such as carcinoma of the stomach, the value of the contribution would be increased if in each case more details were given in the description of the major local pathology.

The appendix contains useful information, such as the average dimensions of adult organs, weight of organs in the newborn, the date of ossification of the principal bones, and the ages of the eruption of the teeth, dates which are well known to every grandmother, but which the majority of doctors are unable to specify.

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*Development of Our Knowledge of Tuberculosis**

HISTORY is one of our greatest sources of instruction. An historical presentation, even one whose scope is as limited as that under review, cannot but be of tremendous interest, particularly when presented in an authoritative manner. Perhaps the most remarkable observation in historical reviews of this sort is how clearly some of our antecedents saw conditions and how epoch-making observations are often lost to sight for years, indeed centuries, to be rediscovered by another who later, to his chagrin, finds himself antedated.

Long before the Christian era the laws of Manu in India declared pulmonary consumption and swelling of the glands of the neck to be unclean, incurable diseases, and cautioned those who desired matrimony not to select a spouse from an unhealthy family, even though the family had good lineage and possessed great wealth.

In 1782 the laws of the kingdom of Naples required that the physician shall report a consumptive case when ulceration of the lungs has been established under penalty of 300 ducats for the first offense and banishment for ten years for repetition of it. Today we still experience difficulty in enforcing the reporting of tuberculosis.

But the chief interest in Dr. Flick's comprehensive review is in his description of the long, laborious, painstaking search for the truth as revealed in the writings of the leading thinkers through the centuries. Often the leaders of medical thought have been very far afield from the truth.

The review terminates with the beginning of the 20th century.

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*Development of Our Knowledge of Tuberculosis. By Lawrence F. Flick, M.D., L.L.D. Cloth. Pp. 783. Price $7.50. 738 Pine St., Philadelphia, Pa. 1925.

The Journal of Laboratory and Clinical Medicine

VOL. XI ST. LOUIS, MO., MAY, 1926 NO. 8

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Richmond, Va.

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Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Appendicitis

EVEN after many years of study of appendicitis the problem of etiology remains. Ever and anon an investigator endeavors to show that the cause of the disease is an unusual organism and attempts to relegate B. coli to the background. But always, it seems, renewed bacteriologic investigations tend to prove that B. coli is, after all, the commonest invader of the appendix and the most important cause of appendicitis. If this is the actual state of affairs, inflammations of the appendix are rarely if ever of hematogenous origin. Aschoff is perhaps the most recent of the so-called authorities to refer appendicitis to unusual causes, and he believes that the streptococcus, either alone or associated with a slender, curved gram-positive bacillus, is the predominating cause. And yet McWilliams found that 57 per cent of 288 cases showed B. coli and only 8.3 per cent showed streptococcus alone.

Warren in a recent article reports the study of 100 appendices from cases in which a diagnosis of acute appendicitis had been made. Of the 100 specimens, only 66 could be diagnosed, by histologic methods, as actually acute. By means of careful bacteriologic methods the organisms invading the walls of the

diseased organs were studied and the results are shown in the following table. The figures apply only to the 66 acute cases.

	SEROSA	MUSCULARIS	MUCOSA	TOTAL
B. coli communis	14	17	18	18
B. coli communior	7	7	7	7
Streptococcus hemolyticus	5	5	5	6
Streptococcus hemolyticus + B. coli	9	9	9	9
Proteus vulgaris	4	4	5	5
B. pyocyaneus	4	4	4	4
B. pyocyaneus + B. coli	1	1	1	1
Streptococcus viridans	3	2	3	3
Streptococcus viridans + B. coli	1	1	1	1
Staphylococcus albus + B. coli	1	1	1	1
Pneumococcus type I	1	1	1	1
Streptococcus (nonhemolytic)	0	0	0	1
No growth	–	–	–	9

There were four cases of periappendicitis, two of which yielded Pneumococcus type I, one Streptococcus viridans, and one the gonococcus.

In 16 cases (in the series of 100) composing the group of "healing" appendicitis, one showed Streptococcus viridans, and one B. coli, both present in the serosa and not in the muscularis. In the remainder of the cases, no growths were secured. This is the group of "healed appendicitis."

The study of this series of cases brought up the problem of tissue diagnosis of surgical specimens as it relates to the appendix. It has been more or less customary for a hospital pathologist to use a very cumbersome series of designations for diagnostic purposes, as, for instance, acute appendicitis, subacute appendicitis, chronic appendicitis, adding to each of these such descriptive words as suppurative, catarrhal, fibrous or fibroid, etc., according as certain appearances in the gross specimen appeal to him. It is obvious that such a nomenclature must vary widely in its actual meaning, and it is also obvious that the diagnosis "chronic appendicitis" is apt to cover not a few surgical misjudgments, and that the term "acute appendicitis" is overworked both by surgeon and pathologist. In the group of 100 cases selected by Warren as *acute* only 66 per cent turned out to be actually acute.

Therefore, as a result of the combined bacteriologic and histologic study Warren suggests the following as the minimum requirements for a diagnosis of the various conditions:

"For acute appendicitis, pus in the lumen and some evidence of inflammation in the wall;

"For healing appendicitis, fairly numerous eosinophiles in the muscularis, also foci of lymphocytes;

"For healed appendicitis, focal collections of lymphocytes in the muscularis or serosa, either inside or outside lymphatics.

"The diagnosis chronic appendicitis is not made. The dividing line between acute and healing appendicitis is not very clear cut, but the lesion is called healing when there is a predominance of eosinophiles over polymorphonuclear leucocytes in the exudate and evidence of proliferation of the fibroblasts. The presence of eosinophiles in the mucosa is considered normal."

These requirements for diagnosis are surely helpful.

—*P. G. W.*

Toxin-Antitoxin Immunization as a Source of Anaphylaxis

PHARMACOLOGY recognizes, in addition to the predominant therapeutic effects of drugs, untoward by-effects not always desirable. It is possible that the practical application of immunology to the prevention of disease must receive a similar consideration.

Stewart,[1] in a recent communication, calls attention to toxin-antitoxin immunization against diphtheria as a factor responsible for an increasing number of individuals, particularly children, sensitized to horse serum and hence liable to anaphylactic reactions.

The increasing use of scarlatinal antistreptococcic serum may serve to increase still further the number of sensitized children so that this possibility must ever be borne in mind when necessity arises for the administration of serum to children as a means of prophylaxis or therapeusis.

Were the anaphylactic manifestations simply those of the usually readily-controlled "serum sickness" the matter would be of relatively minor importance. Stewart, however, encountered seven cases in which the administration of serum for scarlatinal prophylaxis in children previously actively immunized against diphtheria eight to eleven months before was followed by reactions of marked intensity which assumed an alarming degree of severity.

Although no deaths resulted, the occurrence serves as a warning to use care and to remember the possibility.

Active immunization of the child population against diphtheria is so important that its neglect cannot be contemplated. It would seem advisable, however, that studies be directed toward the evolution of some method whereby the production of marked sensitization may be minimized if it cannot be avoided.

It has been suggested by Kolmer that it might be possible to prepare an ox-serum diphtheria antitoxin which could be used for prophylaxis, reserving the horse serum antitoxin for treatment. Some such procedure would, at least, lessen the likelihood of anaphylactic reactions.

Larson and numerous associates[2, 3] have recently presented the results of investigations directly applicable, and so applied by them, to this problem.

During the course of studies upon the effect of surface tension depressants upon bacterial toxins, it was found that when a solution of sodium ricinoleate was brought into contact with a bacterial toxin the toxin was "detoxified." As a result, several hundred times the normal lethal dose could be injected into animals without ill effect.

It was further observed that such detoxified toxins suffered no alteration in their antigenic properties, that the production of immune bodies followed their injection, and that, because of the large dosage possible, immunization occurred very rapidly.

The assumption is that the action of the soap is an adsorption phenomenon and that protection from the toxin occurs by the adsorption of a layer of soap over the entire toxin molecule.

These observations have been applied to the active immunization of human beings, children and adults, against diphtheria and scarlet fever.

It is claimed that when using such detoxified toxins, large doses may be given without either local or general reactions and followed by the production of an active immunity within a short space of time.

If these results are confirmed, a very definite advance will have been made in that active immunization will be possible without an attendant sensitization occurring.

The problem merits serious and extensive study in order that active immunization and the specific serum treatment of disease may not become a source of alarm to the laity because of severe reactions consequent upon the use of these valuable and, indeed, indispensable weapons in the treatment and prevention of disease.

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—*R. A. K.*

Measles

WITH the advent of modern and intensive studies of disease, in particular as well as in general, new concepts take the place of old throughout the entire field of medical practice.

Particular attention has been paid to many of the so-called "children's diseases" which, in olden days, were regarded as necessary and almost unavoidable evils, and many of these, through the acquisition of definite information regarding their etiology and prophylaxis, have more or less been brought under control.

Measles, however, perhaps because sufficiently intensive studies have yet to be made, remains, economically, one of the most important of the diseases of early life.

The deaths from measles in childhood are numbered among the thousands yearly, and the death rate in the first five years of life from bronchopneumonia, so frequently associated with this infection, reaches a total of nearly 50 per cent. In addition, there are no readily available statistics to indicate the degree or extent of the sequelae of measles, such as tuberculosis or conditions affecting the eyes, ears, and other structures.

The disease seems ubiquitous. There is no part of the globe where it does not occur, and it seems to have been known from time immemorial, being held identical with the Sanskrit "masura" (spots).

Definite knowledge of the nature of the etiologic agent is so far lacking, other than that it is present in the secretions of the nose and throat and perhaps the blood, during the first stages of the disease.

Tunnicliff and Moody[1] have described a gram-positive, anaerobic diplococ-

cus obtained from the blood which grew aerobically in the second generation, producing green colonies on an aerobic blood plate.

This organism is stated to have agglutinated with the blood of measles cases from the third to the eighth day after the appearance of the eruption, opsonins also being formed.

While these investigators believe that inoculation into rabbits, guinea pigs, and monkeys was followed by symptoms comparable to measles in the human being, and that these results were due to the coccus in question, an atmosphere of doubt arises from their further statement that "it is possible that the diplococcus possesses the selective power of carrying the specific virus of measles."

Further corroboration of this work has not, apparently, been reported.

The experimental production of measles in the lower animals is a matter of some difficulty, the characteristic picture of the human reaction not having been reproduced.

Scott and Simon[2] review the literature of rabbit infections and report experiments indicating that rabbits react to measles with a marked elevation of temperature and an amphophilic leucopenia, the latter being the more characteristic manifestation in these animals.

The same observers[3] report the occurrence of protective bodies in the blood of rabbits after inoculation with blood or nasopharyngeal secretions from cases of human measles.

There seems to be no doubt that the etiologic agent is present in both the nasopharyngeal secretions and the blood and that protective bodies appear in the blood stream.

Little is known, however, of the pathology of uncomplicated measles, because death is generally due to the accompanying bronchopneumonia or to later complications.

In the cases reported by Denton[4] in which death occurred so soon after onset (within a few hours) that the lesions were probably due to uncomplicated measles, the primary lesions were mainly in the lower trachea, bronchi, and contiguous lung and were in the nature of an acute, congestive inflammatory reaction without distinctive characteristics.

Because of the lasting immunity following attacks, convalescent serum has been used with encouraging results in its treatment and prophylaxis.

Regan[5] reports that the symptomatology was much modified in immunized individuals later developing the disease, and McNeal[6] believes this to be an efficacious prophylactic measure.

Zingher[7] has used the method rather extensively and has shown that either plasma, serum, or whole blood (citrated or noncitrated) may be used, the resulting immunity persisting from thirty to forty-five days.

Weaver and Crooks[8] believe that prophylaxis through the injection of convalescent serum should be practiced in children under the age of five.

The method, while of undoubted efficacy, is handicapped by the difficulty of obtaining a sufficient supply of serum and the effective specific prophylaxis and treatment of this disease awaits the demonstration of its specific agent.

The problem merits persistent and organized study.

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—*R. A. K.*

CORRESPONDENCE

19th December, 1925.

To the Editor:

In connection with the paper by Dr. Anderson on the "Use of a Suction Apparatus for Bleeding Rabbits" which appeared in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE for November, 1925, it may be of interest to note that a similar, indeed almost identical, apparatus was introduced for the same purpose in the Wellcome Tropical Research Laboratories at Khartoum prior to 1911. An account of it, with illustrations of the apparatus, will be found in the Fourth Report of these Laboratories, Vol. A., p. 107. I may say the apparatus was primarily introduced for the purpose of preventing contamination of blood-agar tubes, which is very likely to occur in a climate like that of Khartoum, but the arrangement was also found of use in the manner indicated by Dr. Anderson of Toronto, i. e., a larger amount of blood could be obtained from each rabbit by this method than was possible by any other means.

Andrew Balfour,
Director, London School of Hygiene
and Tropical Medicine.

Jan. 25th, 1926.

To the Editor:

The publication referred to in Dr. Balfour's letter is not available for perusal in the city of Toronto, and I am therefore unable to verify the claims regarding priority of publication of a suction apparatus for bleeding small animals. I am, however, pleased to learn that Dr. Balfour endorses the use of an apparatus of this type.

C. M. Anderson,
Director of Laboratories, Ontario
Department of Health.

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